Response of rainbow trout (*Salmo gairdneri*) blood gas transport system to temperature, oxygen availability and photoperiod

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ABSTRACT

Hematological status in rainbow trout, Salmo gairdneri, was examined in relation to eight combinations of three environmental factors; temperature (5°, 20°C), oxygen availability (<35%, >70% saturation) and photoperiod (16L:8D, 8L:16D) and evaluated by 3-factor analysis of variance. Hemoglobin and hematocrit, indicators of oxygen-carrying capacity increased significantly at the higher temperature, following exposure to hypoxia and in relation to reduced light period. Significant variations in mean corpuscular hemoglobin concentration were not detected. The effects of temperature and oxygen availability were more pronounced than that of photoperiod which was generally masked. Although oxygen availability and photoperiod did not interact with temperature, the interaction of the former factors was significant. Electrophoresis revealed twelve hemoglobin isomorphs. Relative concentration changes were found in relation to the factors considered with temperature>hypoxia>photoperiod. However, in terms of absolute concentration, effects were hypoxia>temperature>photoperiod. Photoperiod effects were again masked by temperature and (or) hypoxia. Red cell levels of $[Cl^-]$ and $[Mg^{+2}]$, critical elements in the hemoglobin-oxygen affinity regulating system, were also significantly altered. Red cell Cl^- was influenced only by temperature; Mg^{+2} by temperature and oxygen. No photoperiod influence on either ions was observed. Under nominal 'summer' conditions, these changes point to the likelihood of increases in oxygen-carrying capacity coupled with low Hb-O_2 affinity adjustments which would be expected to increase oxygen delivery rates to their more rapidly metabolising tissues.

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INTRODUCTION

North temperate zone aquatic habitats are characterized by well-defined seasonal variations in environmental conditions (e.g. temperature, oxygen availability, pH, photoperiod) and during the summer months by significant daily fluctuations. Their endemic fauna, including fishes, can be significantly affected by these physical and chemical as well as accompanying biological changes, and commonly exhibit compensatory responses of one kind or another.

The principal physical factors involved include temperature, light, water movements and levels, water density, and substrate characteristics. Chemical factors include dissolved gases, inorganic and organic ions, and nonionizable materials. Of these temperature, oxygen and photoperiod are highly variable with respect to season, and biologically effective. Temperature, for example, exhibits not only seasonal variations, but in lakes and ponds, and to a lesser extent, rivers and streams, also vertical variation as well. Thus, fishes which migrate vertically for feeding may pass through a substantial thermal range even though the thermal characteristics of their habitats are stable. For example, the juveniles of sockeye salmon, Oncorhynchus nerka, a relatively stenothermal species, may expose themselves to temperature changes of 10^{o}C or more per day in this way (Biette and Geen, 1980), and this represents about 35% of their thermal tolerance zone.

As water temperature increases, the availability of oxygen decreases because of thermal effects upon the solubility of oxygen (Henry's law). Temperature-induced increases in the metabolic rates

of micro- and other coexisting organisms frequently further reduce oxygen levels. Fishes, with few exceptions (e.g. tunas, lamnid sharks: Fry and Hochachka, 1970; Stevens and Neill, 1978) are ectotherms, and are characterized by body temperatures which are normally within less than one degree of ambient water temperature (Stevens and Sutterlin, 1976). Consequently, their metabolic rates are sharply influenced by water temperature conditions (Figure 1). Q10 values for oxygen uptake rates by fish are frequently in the 2.0-2.3 range, but can be much higher (Fry and Hochachka, 1970). In the case of rainbow trout, Salmo gairdneri, the species employed in this study, oxygen consumption increased from 18.7 to 121.4 $mg \cdot kg^{-1} \cdot hr^{-1}$ over a 2° to 18°C range, while oxygen content declined from 13.8 to 9.4 $mg.l^{-1}$ (Henry and Houston, 1984). In the relatively eurythermal goldfish, Carassius auratus, oxygen uptake was enhanced by 254% when temperature was changed from 10° to 20°C (Fry and Hart, 1948).

Oxygen demand in teleost can be related to temperature by a parabolic function, the Bělehradek metabolism-temperature (MT) relationship:

$$\dot{V}O_2 = KoT^{k1}$$

or its linear equivalent, the Krogh relationship:

$$\dot{V}O_2 = Ko + K_1T$$

where, $\dot{V}O_2$ = oxygen consumption

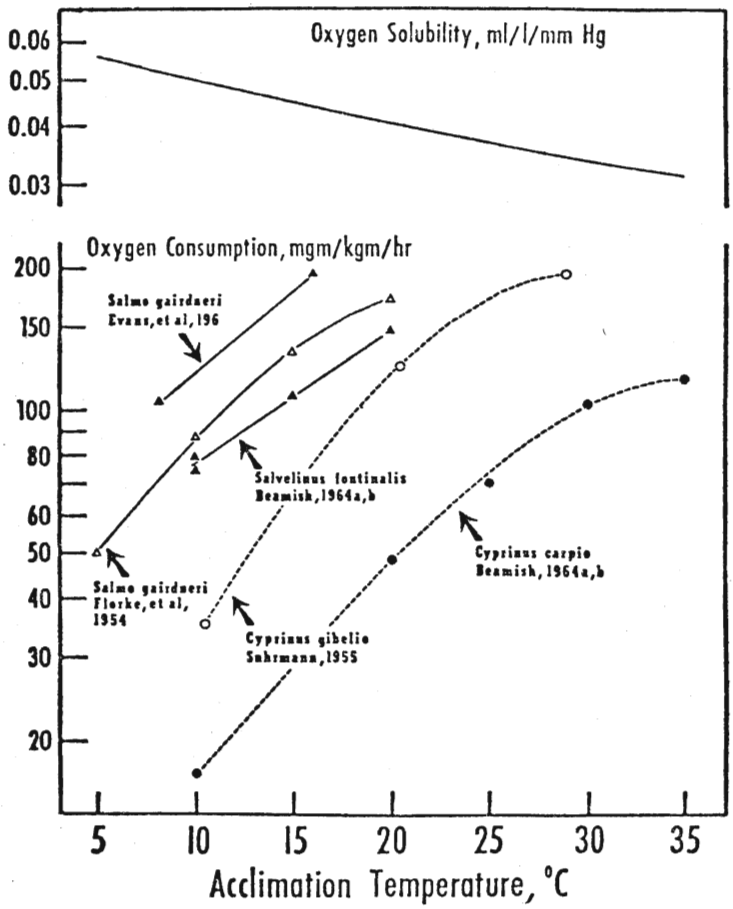
T = temperature

Ko = limiting value (i.e., $\dot{V}O_2$ at lowest tolerated temperature)

K_1 = slope (van't Hoff Q10)

In short, increases in environmental temperature confront these animals with the problem of satisfying increased oxygen requirements under conditions of diminished oxygen availability. Responses to temperature-related respiratory stress can be complex, with manifestations

Figure 1 Variations in oxygen solubility ($ml.l.^{-1}.mmHg^{-1}$) and oxygen demand ($mgm.kgm^{-1}.hr^{-1}$) in some representative teleosts with water temperature (Houston, 1973)


Oxygen Solubility, ml/l/mm Hg
0.06
0.05
0.04
0.03
Oxygen Consumption, mgm/kgm/hr
200
150
100
80
60
50
40
30
20
Salmo gairdneri
Evans, et al, 196
Salvelinus fontinalis
Beamish, 1964a, b
Salmo gairdneri
Florke, et al,
1954
Cyprinus gibelio
Suhrmann, 1955
Cyprinus carpio
Beamish, 1964a, b
5
10
15
20
25
30
35
Acclimation Temperature, °C

seen at virtually all levels from the behavioural to the molecular. In many species, however, the most prominent features of response are those which involve the branchial oxygen-carbon dioxide transfer system and blood oxygen transport. In the present study, emphasis has been given to responses at the hematological level. These are conveniently grouped within the general scheme proposed by Hochachka and Somero (1973).

(1) Adjustments in overall oxygen-carrying capacity (the so-called quantitative adaptive strategy).

(2) Adjustments in the types or abundancies of the hemoglobins present in species possessing functionally-distinct hemoglobins (the qualitative adaptive strategy).

and (3) Adjustments in the microenvironment in which these hemoglobins operate, particularly with respect to factors influencing hemoglobin-oxygen affinity, and therefore the readiness with which oxygen is loaded at the gills and subsequently unloaded in the microcirculation (the modulatory adaptive strategy).

Earlier studies in this laboratory (Houston and DeWilde, 1968; 1969; Houston and Cry, 1974; Houston et al, 1976; Houston and Smeda, 1979; Houston and Koss, 1984a,b; Henry and Houston, 1984) have emphasized responses to temperature under normoxia, i.e., near-saturation (>80%) oxygen levels. In these studies, the higher temperature conditions were accompanied by hypoxia only in the sense that the amount of oxygen available may have been inadequate to ensure full hemoglobin loading or to satisfy temperature-enhanced tissue needs. All previous experiments were also carried out under what are regarded as "neutral" photoperiod conditions, i.e. 12 hours light and 12 hours darkness per day. Actually, under natural conditions reduced temperatures and high oxygen availability are associated with short light periods,

i.e. 'winter' conditions, whereas the reverse is true during the summer period. In nature, photoperiod variations can have two potentially important effects. Oxygen variations, especially in shallow freshwater habitats are at least partly the result of diurnal changes in net oxygen production by photosynthesis. It has been shown, for example, that PO_2 may drop to 20-30 mmHg during the night, and rise to 200-400 mmHg at mid-day as a consequence of this (Jones, 1961; Garey and Rahn, 1970; Davis, 1975; Kramer et al, 1978). Photoperiod also has significant effects upon endocrine activity. These are thought to be mediated by the pineal gland, and have a wide range of effects. Murphy and Houston (1977), for example, have demonstrated significant photoperiodic effects on water-electrolyte metabolism in rainbow trout. As yet, however, possible photoperiodic influence upon oxygen-carrying capacity have not been examined.

In the present study, consideration has been given to the effects of temperature acting in concert with oxygen availability and photoperiod, on the amounts, kinds and operating conditions of hemoglobin in the rainbow trout, Salmo gairdneri.

The Null hypothesis proposed was "temperature, oxygen availability and photoperiod do NOT influence:

(1) total blood hemoglobin content and hematocrit

(2) the qualitative and quantitative organization of the multiple hemoglobin system

and (3) erythrocytic levels of the hemoglobin-oxygen affinity modulators, Mg^{+2} and Cl^{-1}."

REVIEW OF LITERATURE

The following Review of Literature focuses on three principal areas. The first of these is concerned with cardiovascular-ventilatory-branchial aspects of response to stresses of the types considered in this study. Although, as noted previously, this investigation emphasizes hematological aspects of response, these do not occur in isolation. Maximum rates of oxygen delivery to tissues are established by several factors. The degree to which hemoglobin is oxygenated, for example, during passage of blood through the gills is influenced by effective branchial area and rates of ventilatory and perfusion flow as well as the amount of hemoglobin available for oxygen uptake. Delivery of oxygen to tissues is essentially a product function of cardiac output, which approximates branchial perfusion in linear circulatory systems, and blood oxygen-carrying capacity. The final step, unloading of oxygen within the microcirculation is, on the other hand, regulated by the modulatory microenvironment provided by the erythrocytes. In short, hematological responses must be viewed in the context of systemic activities. Accordingly, attention has initially been given to this question, and particularly to the advantages and limitations of various systemic responses.

A subsequent section considers the structural and functional properties of hemoglobin, and factors involved in establishment of the equilibrium between the 'tense', deoxy- and 'relaxed', oxy-configurations of the molecule. This has been done primarily by reference to the intensively-studied mammalian hemoglobin system. Where possible, however, reference has been made to comparable properties in teleostean hemoglobins and, in addition, to what appear,

by reference to the mammalian situation, to be their unique properties.

The final section of this review addresses hematological responses to stresses similar to those investigated in this study.

I. Adaptations of the Cardiovascular-Respiratory System

Salmonid fishes normally inhabit temperate, well-oxygenated waters. As noted in the Introduction, they may be subjected to stresses of various kinds as a consequence of variations in their environment, and must then respond with compensatory adaptations to their altered circumstances. Although variations in temperature and oxygen availability are frequently linked under natural conditions, most of the research which has been reported thus far has considered adaptive response to either temperature or oxygen content. Responses at the respiratory level involve (1) ventilation: $\dot{V}g$, the volume of water passing over the gills per unit of time, (2) perfusion: $\dot{Q}g$, the volume of blood passing through the gas exchange area per unit of time (this is usually symbolized as $\dot{Q}g$ cardiac output - the product of cardiac stroke volume and heart rate - since most of the blood ejected by the heart is delivered to the gills) and, (3) diffusion, which is a function of gas exchange area (A), mean diffusion pathway ($\Delta\chi$) and permeability (P). The relationships of these variables to oxygen uptake ($\dot{V}_{O_2}$) have been described by Hughes and Shelton (1962), Hughes (1964), Randall et al (1967) and Randall (1970) in terms of three basic respiratory equations:

(1) Branchial ventilation : $\dot{V}_{O_2} = \dot{V}g \cdot \alpha W_{O_2} \cdot (PI_{O_2} - PE_{O_2})$ ml $O_2 . min^{-1}$

(2) Branchial perfusion: $\dot{V}_{O_2} = \dot{Q}g \cdot \alpha b_{O_2} \cdot (Pa_{O_2} - Pv_{O_2})$ ml $O_2 . min^{-1}$

(3) Branchial diffusion: $\dot{V}_{O_2} = (dA/2\Delta\chi) \cdot (PI_{O_2} - Pa_{O_2} + PE_{O_2} - Pv_{O_2})$

ml $O_2 . min^{-1} . cm^{-2}$.

where: $\dot{V}_{O_2}$ = oxygen consumption (oxygen uptake)

αW_{O_2} = water oxygen capacity

αb_{O_2} = blood oxygen capacity

$\dot{V}g$ = ventilatory water flow over the gills

$\dot{Q}g$ = cardiac output (i.e. blood flow through the gills)

PI_{O_2} = oxygen tension of inspired water

PE_{O_2} = oxygen tension of expired water

Pa_{O_2} = arterial oxygen tension

Pv_{O_2} = venous oxygen tension

d = coefficient of oxygen diffusion

A = gill exchange area

$\Delta\chi$ = mean diffusion path length

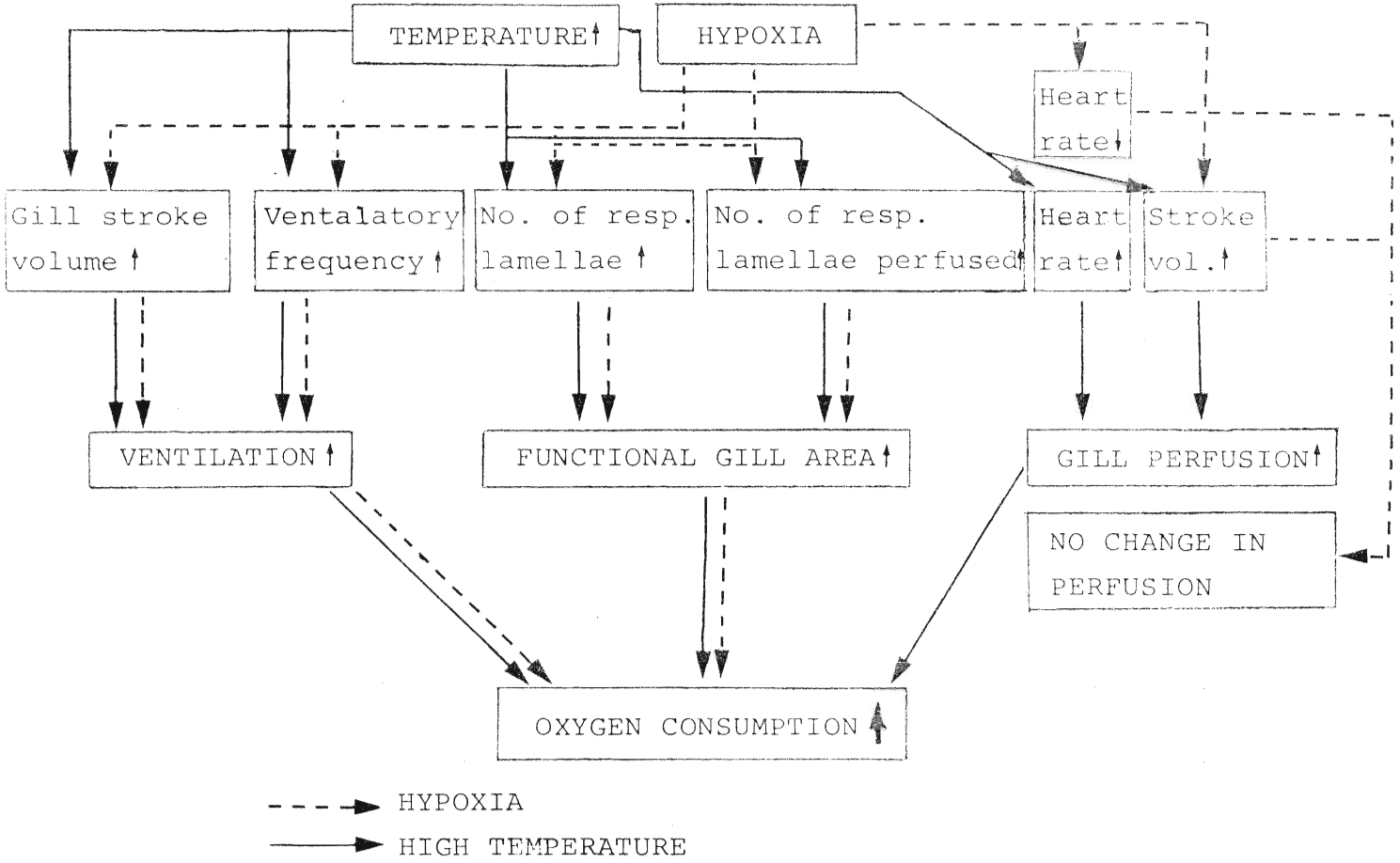
While some studies have emphasized the effects of abrupt changes in water temperature (Wells, 1935; Freeman, 1950; Grainger, 1958; Sage, 1971; Crawshaw, 1976) most have focused on the influence of long-term thermal acclimation processes (Hughes, 1970; Heath and Hughes, 1973; Cech et al, 1979; Biette and Geen, 1980; Henry and Houston, 1984). This has considerable bearing on the results obtained since it has been well established that the changes in oxygen consumption which occur in fish when temperature is altered depend to some extent on the rate as well as the magnitude of the imposed change. In addition, response to such changes also depends upon the previous thermal history of the fish (Holeton, 1979).

Patterns of oxygen consumption are usually profoundly altered immediately following abrupt temperature change and stabilize during acclimation to the new temperature conditions for a few days (Bullock, 1955). In nature, however, neither abruptly changing temperatures nor long-term constant temperatures are commonly encountered. On the other hand, many aquatic organisms are exposed to a diurnal

thermal cycle as a result of vertical or horizontal movement and/or because of the thermal characteristics of their habitats (Henry and Houston, 1984). A number of recent studies have, therefore, examined respiratory responses to the more ecologically-realistic circumstances provided by regularly-fluctuating temperatures, comparing these with responses observed under conditions of constant temperature.

Similarly, cardiovascular-respiratory responses to gradually-imposed hypoxia have now been described (Marvin and Heath, 1968; Hughes and Saunders, 1970; Burton, 1971; Holeton, 1971) and supplement earlier studies involving abrupt exposures to reduced oxygen tension. Because the responses of the cardiovascular-respiratory system to temperature changes and hypoxic conditions share a number of common features, it is convenient to review them together (Figure 2).

Figure 2 Systemic adaptation in teleosts to thermal and hypoxic stresses


TEMPERATURE↑
HYPOXIA
Heart rate↓
Gill stroke volume↑
Ventalatory frequency↑
No. of resp. lamellae↑
No. of resp. lamellae perfused↑
Heart rate↑
Stroke vol.↑
VENTILATION↑
FUNCTIONAL GILL AREA↑
GILL PERFUSION↑
NO CHANGE IN PERFUSION
OXYGEN CONSUMPTION↑
HYPOXIA
HIGH TEMPERATURE

Temperature-induced increases in oxygen consumption are commonly associated with elevated gill ventilation (Wikgren, 1953; Davis, 1968; Hill and Potter, 1970; Heath, 1973; Johansen et al, 1973; Henry and Houston, 1984). This type of response has been observed in a variety of fish species including the largemouth bass, Micropterus salmoides (Reynolds, 1977), goldfish, Carassius auratus (Beamish and Mookherjii, 1964), flounder, Platichthys flesus (Duthie and Houlihan, 1982) and rainbow trout, Salmo gairdneri (Hughes and Roberts, 1970). A highly significant correlation between oxygen consumption and ventilatory flow was recently reported for rainbow trout in this laboratory (Henry and Houston, 1984). In this study, ventilatory flow was enhanced some 5.50 x over a temperature range of 2^{o} to 22^{o}C. This resulted from increases in both ventilatory frequency and stroke volume which were elevated by 2.57 and 2.36 x respectively. A dependency of ventilatory frequency upon ambient temperature has been reported in numerous other species of fishes including the carp, Cyprinus carpio (Meuwis and Heuts, 1957), goldfish (Freeman, 1950) and guppy, Lebistes reticulatus (Tsukuda, 1961). Interestingly, no evidence of respiratory acclimation to diurnal cycling temperature conditions per se has been observed (Duthie and Houlihan, 1982; Vondracek et al, 1982; Henry and Houston, 1984), and this stands in distinct contrast to other responses to such circumstances.

The respiratory responses of fishes have also been examined in some detail following imposition of both moderately and acutely hypoxic conditions (Holeton and Randall, 1967; Cech and Wohlschlag, 1973; Johansen, 1982; Lomholt and Johansen, 1979; Nikinmaa and Weber, 1984).

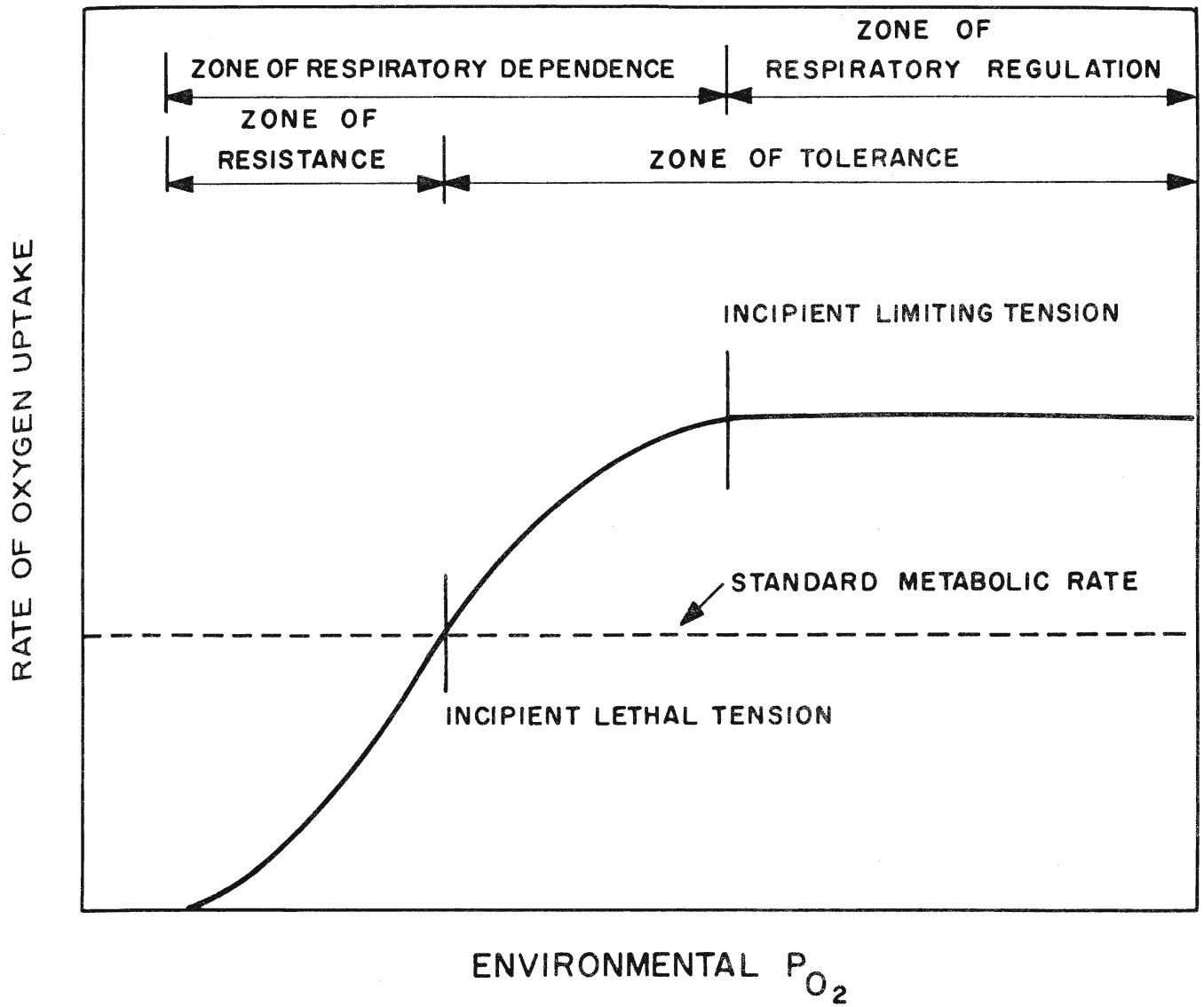
For example, in rainbow trout ventilatory frequency rises from 86 to 123 cycles.min^{-1} when Po_2 is decreased from 140 to 30 mmHg (Holeton and Randall, 1967). Similarly, in the lamprey, Lampetra fluviatilis a decrease in Po_2 from near-saturation levels to 40 - 50 mmHg prompts a more than two-fold increase in ventilation rate, i.e., from 99 to 241 cycles.min^{-1} (Nikinmaa and Weber, 1984). This is true as well of the related lamprey, Entosphenus tridentatus (Johansen et al, 1973). These animals exhibit increases in gill stroke volume as well as ventilatory rate in response to hypoxia. This has also been reported in rainbow trout (Hughes and Saunders, 1970; Davis and Cameron, 1971), the lung fish, Neoceratodus forsteri (Johansen et al, 1967) and striped mullet, Mugil cephalus (Cech and Wohlschlag, 1973).

As a consequence of increases in stroke volume and ventilation rate, ventilatory flow is sharply elevated with hypoxia. Cech and Wohlschlag (1973), for example, reported that mean ventilatory flow increased more than 3-fold in the mullet. This was achieved by an approximate doubling of the mean stroke volume and a proportionately smaller increase in ventilatory frequency. Their findings tend to support the contention of Johansen et al (1967) that " . . . there may be an energetic advantage in raising ventilatory flow by increases in ventilatory stroke volume rather than frequency. . ." It is interesting to note that although both ventilatory frequency and stroke volume rise in response to moderate hypoxia, severely hypoxic conditions lead to decreases in both ventilatory frequency and stroke volume and, thus, a decline in ventilatory flow (Beamish, 1964; Gerald and Cech, 1970;

Holeton, 1971; Marvin and Burton, 1973; Nikinmaa and Weber, 1984). Simulation studies by Taylor et al (1968) suggest that this reversal of the normal response to hypoxic conditions may stem from a braking reflex brought into play when the triggering stimulus exceeds some maximum limit. They hypothesized that this limit represents a stimulus magnitude which would force the ventilatory system to a level of metabolic cost in excess of the additional gain in oxygen uptake. In short, the intervention of the suggested braking reflex prevents the ventilatory mechanism from operating as a positive feedback system.

The relationship of oxygen consumption to oxygen availability is frequently described in terms of zones of respiratory dependence and independence (Van Dam, 1938; Fry, 1947; Hughes, 1981)(Figure 3). These are defined primarily in terms of maximum, rather than standard or basal levels of oxygen uptake. Over a range of relatively high, species-dependent oxygen tensions, oxygen consumption at constant temperature remains stable. The range of oxygen tensions over which consumption is not influenced by availability is said to constitute the zone of respiratory independence. The response mechanisms which permit the animals to maintain stable oxygen consumption rates under these circumstances involve the circulatory and ventilatory systems and are considered in more detail later in this review. However, as oxygen tension falls to a particular level, the critical oxygen tension (Tc), oxygen uptake declines as Po_2 decreases despite responsive increases in cardiovascular-respiratory activities. Since the metabolic costs of operating these systems rise rapidly with increased activity, the actual net gain in oxygen uptake probably falls more rapidly than does overall oxygen consumption itself. In any event, the range of

Figure 3: The relation between standard and active (maximum) oxygen uptake rates at different environmental oxygen concentrations (From Hoar, 1966).


ZONE OF RESPIRATORY DEPENDENCE
ZONE OF RESPIRATORY REGULATION
ZONE OF RESISTANCE
ZONE OF TOLERANCE
INCIPIENT LIMITING TENSION
STANDARD METABOLIC RATE
INCIPIENT LETHAL TENSION
RATE OF OXYGEN UPTAKE
ENVIRONMENTAL P_{O_2}

tensions over which a direct relationship is seen between oxygen consumption and availability is termed the zone of respiratory dependence. This has a lower boundary, the incipient lethal tension; sometimes referred to as the tension of no excess activity. This is the tension at which maximum and basal levels of oxygen consumption intersect, i.e., at tensions below the incipient lethal level the animal begins the process of asphyxic death. This has been well documented in a number of species including brook trout, carp and goldfish (Beamish, 1964). Changes in ventilation also affects % utilization (% U) of oxygen (Van Dam, 1938; Gerald and Cech, 1970). The basis of decreasing % U with increasing ventilation will be considered later. As was the case with response to temperature change, the extent of ventilatory response following exposure to hypoxia is time dependent. With the onset of acutely hypoxic conditions ventilatory frequency, stroke volume and oxygen consumption initially increase rapidly and markedly (Hughes and Saunders, 1970; Johansen et al, 1973; Claridge and Potter, 1975). With continued exposure, however, both ventilatory frequency and flow decrease, as noted earlier, although they remain well above the levels found under normoxic conditions. Nikinmaa and Weber (1984) have recently demonstrated this phenomenon in the lamprey and their observations are summarized in Table following.

Table 1

Ventilatory responses to acute and prolonged hypoxia in Lamprey (*Lampetra fluviatilis*) at 15°C

Reported as mean± standard error of the mean (sample number)

(Data from Nikinmaa and Weber, 1984)

Parameter	Normoxia	Acute Hypoxia	7 days Hypoxia
Pwo_2 (mmHg)	>130	40-50	40-50
Ventilatory frequency ($cycle.min^{-1}$)	99 ± 14 (8)	241 ± 37 (8)	224 ± 40 (8)
O_2 consumption ($mg.kg^{-1}.hr^{-1}$)	40 ± 3.7 (8)	60 ± 5.2	53 ± 3.5

Nikinmaa (1981) and Weber (1982) have hypothesized that responses to prolonged hypoxia represent adaptations involving energetically less costly adaptive mechanisms; among them enhanced oxygen-loading in gills as a consequence of increased hemoglobin-oxygen affinity. This has also been reported in eels, *Anguilla anguilla* and *Lampetra fluviatilis* (Wood and Johansen, 1972; Nikinmaa and Weber, 1984), Plaice, *Pleuronectes platessa* (Wood *et al*, 1975), carp (Weber and Lykkeboe, 1978) and rainbow trout (Soivio *et al*, 1980; Tetens and Lykkeboe, 1981; Nikinmaa and Soivio, 1982). The mechanism which increases the Hb-oxygen affinity and oxygen-carrying capacity will be reviewed in later section.

Although increased ventilatory activity is commonly employed by terrestrial vertebrates to amplify oxygen uptake, a number of considerations appear to limit its utility in aquatic organisms including fishes. Among the most important of these is the nature of the respiratory medium. This is, as previously noted, dense, viscous and relatively oxygen-poor. Furthermore, hydrodynamic considerations ensure that not all of the water delivered to the gills is used for respiratory purposes. Because of these characteristics, gill-breathing animals require a relatively massive ventilatory musculature, and must expend substantial energy in ventilation. In fishes, for example, as much as 30% of total metabolism may be directed to the operation of the muscular pumps responsible for ventilation (Hughes and Shelton, 1962; Schumann and Piiper, 1966). Although precise figures are not available, the cost of ventilation varies depending on species, activity and characteristics of the habitats. Normally, only 30-40% of the oxygen presented to the gill surfaces is actually extracted. This is a consequence of relatively large diffusion resistances across the gill surface and also because of water shunting. The latter constitutes the proportion of ventilatory flow that does not come into contact with the respiratory surface of the gills, being shunted past the gills. Randall (1970) has summarized the paths which water may take through the branchial chamber, distinguishing respiratory flows, shunt flows and residual flows. The shunt flow, in turn, can be subdivided into three components. When interlamellar spaces are large or water flow is so fast that there is not sufficient time for oxygen extraction, a 'diffusional dead space' results. Secondly, there is a 'distributional dead space' as a

consequence of unequal ventilation and perfusion of the gills. If ventilation through lamellar "pores" is high, or if the "pores" are ventilated unequally, more oxygen may be delivered to all or part of the respiratory surface than is required to saturate the blood. The final component is the 'anatomical dead space flow'; the water flowing between the primary lamellae of adjacent hemibranchs. The actual water shunt can be calculated as:

$$\text{water shunt} = \frac{\dot{V}g(PE_{O_2} - Pveq_{O_2} - \Delta P_{O_2})}{PI_{O_2}}$$

where, $\dot{V}g$ = ventilatory flow

PE_{O_2} = oxygen tension in expired water

PI_{O_2} = oxygen tension in inspired water

$Pveq_{O_2}$ = oxygen tension in water having the same P_{O_2} as venous blood

ΔP_{O_2} = oxygen gradient between blood and ventilated water

In trout, shunt flow may account for up to ∿ 60% of total ventilation flow under some circumstances (Stevens and Randall, 1967). Generally, water leaving the gills is between 60-70% saturated with oxygen. As noted earlier, increases in temperature lead to increased ventilatory flow. One consequence of this is an increase in anatomical dead space flow, so that more water is shunted past the tips of the gill filaments without passing over the secondary lamellae (Hughes and Morgan, 1973). Consequently, the percentage utilization of oxygen (% U) is reduced following increased ventilation unless other factors intervene. It is widely accepted, for example, that an increase in the

functional lamellar area, or an alteration of blood flow and its distribution in lamellae can come into play to compensate for this effect.

The functional surface area of the gill is defined in the first instance, as the total area of the secondary lamellae; the primary filaments and other branchial areas do not participate in gas transfer. Mean secondary lamellae area, calculated for several species of fishes, is about 4.9 $cm^2.g^{-1}$ body weight (Gray, 1954; Hughes, 1966). However, active fishes have larger areas than do more metabolically-sluggish forms (Gray, 1954; Hughes, 1966). The metabolically-active salmonids, for example, are characterized by relatively large areas per unit body weight than are many other species, and their gills are considerably more effective in the oxygen uptake (Hughes, 1966). Exchange areas calculated in this way are, however, primarily of relative rather than absolute value since allowance must be made for the columnar portions of the pillar cells which separate the lamellar walls. The distribution of these is species-specific, and the actual exchange area (total secondary lamellar area minus cross-sectional pillar cell area) must be calculated for each species.

In order to minimize endosmosis and inorganic ion losses, fresh-water teleosts do not ordinarily utilize all of the exchange area under resting conditions. Simulation studies (Taylor et al, 1968) suggest, for example, that much less than 1/3 of the total is actually employed by trout under such circumstances. Consequently, additional area can be recruited as needed, although at osmoregulatory cost. Such increases in functional area may be accomplished by altering the distribution of branchial blood flow to non-exchangeable versus exchangeable areas of the gill or by amplifying the number of lamellae perfused. Steen

and Kruysse (1964) described three main blood flow paths connecting the afferent and efferent filamental arteries. Based on their demonstration of by-pass shunts, Steen and Kruysse (1964) hypothesized that changes in diffusion capacity could be explained by variations in the proportion of the functional blood flow allocated to non-respiratory vascular shunts. Although such gill blood shunts, which bypass the lamellae were reported in eel (Steen and Kruysse, 1964), channel catfish (Boland and Olson, 1979), elasmobranchs (Piiper and Baumgarten-Schumann, 1968) and Dipnoi (Johansen and Hanson, 1968), later studies have failed to find these shunts in other species (Gannon et al, 1973; Cameron, 1974; Laurent and Dunel, 1976; Vogel et al, 1973, 1974, 1976; Cooke and Campbell, 1980).

Subsequently, an infrared photographic technique was introduced by Davis (1972) to examine gill vascularization. Differences in blood flow to the lamellar blood vessels were observed in adrenalin-treated as compared to control trout. Adrenalin reduces vascular resistance in gills. These and later studies indicated that increases in functional gill surface area were achieved principally by varying the number of secondary lamellae receiving blood. This was first reported by Cameron (1974) and confirmed by Booth (1979) and Petterson and Johansen (1982) for Arctic grayling, Thymallus arcticus, burbot, Lota lota and common sucker, Catostomus catostomus, rainbow trout and Atlantic cod, Gadus morhua respectively. More recently, Petterson and Johansen (1982) have examined mechanisms by means of which this occurs. Apparently, oxygen-sensitive, smooth muscle elements in efferent lamellar arterioles respond to hypoxia by contraction. This vasocontraction increases afferent lamellar pressure causing perfusion of previously unperfused lamellae and hence, increases the functional surface area.

Adjustments in cardiac output (the product of heart rate and cardiac stroke volume) also contribute to respiratory adaptation; the increases

in ventilation which accompany exposure to higher temperatures being associated with increases in cardiac output (Randall, 1968). The metabolic requirements for cardiac pump operation have not been precisely defined in fishes yet, but are thought to be less than those associated with ventilation (Jones, 1971). Cardiac rate varies directly with temperature up to a critical level which is a characteristic of the species, and above this declines (Mott, 1957). For example, in rainbow trout, maximum cardiac rate was recorded at 18°C and did not increase significantly above this temperature (Henry and Houston, 1984). Increases in cardiac rate with increasing temperature have also been reported in other species such as Ophiodon elongatus (Randall, 1968) and Opsanus tau (Wilber, 1961). It is believed that temperature acts directly on the pacemaker cells of the heart, altering membrane permeability and increasing intrinsic rates of depolarization in these cells (Laurent, 1962). This was demonstrated by Randall (1968) for lingcod, and can be inferred from observation on rainbow trout (Henry and Houston, 1984).

In addition, temperature-related variations in ECG intervals suggest that both the rate of spread of the excitation wave through the myocardium, and the rate of recovery of these cells following contraction are increased at higher temperatures (Henry and Houston, 1984). Surprisingly, stroke volume does not appear to be greatly influenced by temperature. This has been demonstrated by Randall (1968) for lingcod, and can also be inferred from observations on rainbow trout (Henry and Houston, 1984). Under such circumstances, increases in cardiac rate at higher temperatures must lead to an enhancement of cardiac output. Since most of the blood ejected by the heart passes through the ventral aorta to the afferent branchial arteries, branchial perfusion must also rise. However, whether this leads to increases in

effective gas exchange area will depend upon the intervention of lamellar responses of the type previously considered. In the instance of the rainbow trout, recent evidence of increased oxygen utilization at higher temperatures suggest that both branchial perfusion and exchange area are elevated (Henry and Houston, 1984).

Although, as noted earlier, there are commonalities in ventilatory responses to both temperature-induced increases in oxygen consumption and to reduced oxygen availability, this is frequently not the case with cardiovascular response. Teleosts frequently respond to hypoxic conditions with reductions in cardiac rate, i.e., bradycardia (Holeton and Randall, 1967 ; Holeton, 1971). Furthermore, in trout, the bradycardia induced by moderately hypoxic circumstances is associated with significant increases in stroke volume. Consequently, there is little change in cardiac output (Holeton and Randall, 1967). It is thought that the combination of reduced cardiac rate and elevated stroke volume may significantly alter the transit time of blood through the gills. Since ventilatory flow volume rises while perfusion flow volume is reduced, improved branchial oxygen uptake would be anticipated (Satchell, 1961; Hughes and Shelton, 1962; Hughes, 1964).

II. Structural and Functional Properties of Hemoglobin

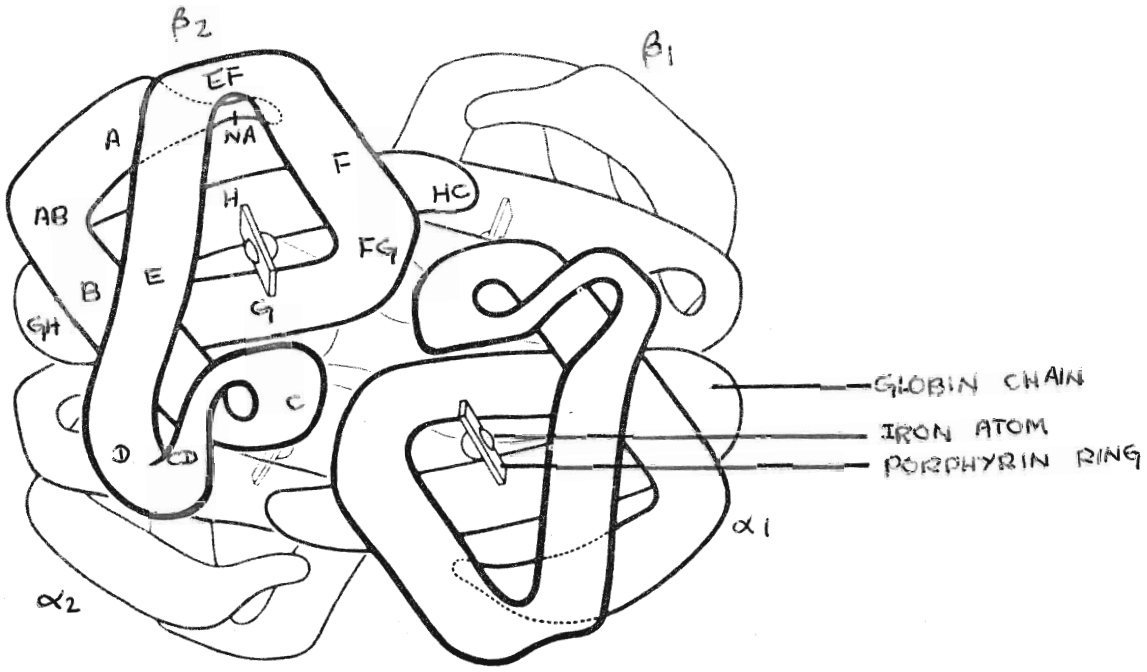
The hemoglobin molecule exhibits well-described structure-function relationships and ligand-related changes in both tertiary and quaternary configurations (Perutz, 1969, 1970) . The primary amino acid sequences of the globin component of the molecules have been determined primarily by reference to mammalian species. However, partial sequences for fishes such as carp (Hilse and Braunitzer, 1968), trout (Bossa et al, 1978), lamprey, Lampetra fluviatilis (Braunitzer and Fujiki, 1969) and and desert sucker, Catostomus clarkii (Powers and Edmundson, 1972a,b) have been determined. These share a number of common features with

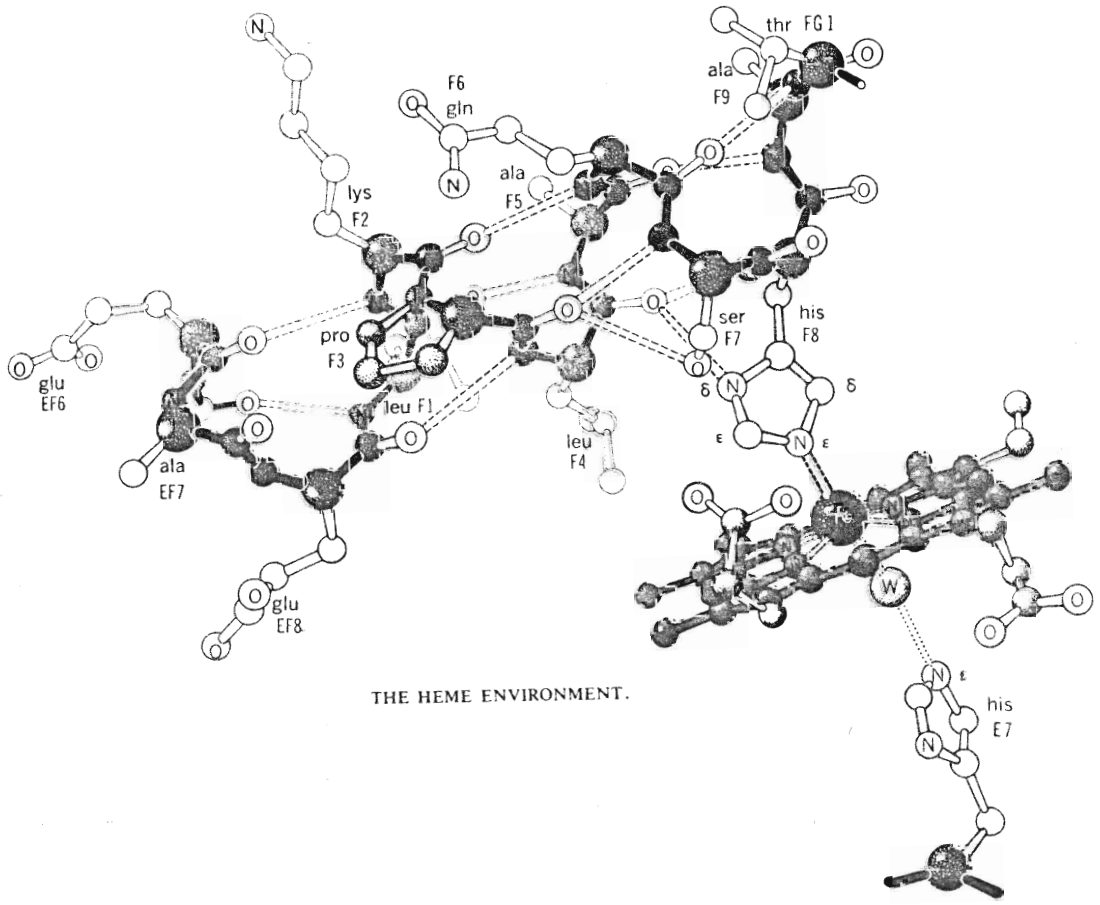
human and other mammalian hemoglobins. In general, vertebrate hemoglobins are remarkably uniform in molecular weight (∿65,000) as well as with respect to the amino acid sequences of their globin chains (Riggs, 1970). This is particularly true of residues which are critical to the oxygen-binding process. For example, two histidines in each globin chain are found at 58 (61 in trout HbI) and 87 (90 in trout HbI) residue sequence positions in both human and fish hemoglobins.

The hemoglobin molecule can be regarded, in some aspects, as a "pseudoenzyme". Like enzyme proteins, it possesses a specific, tightly-bound prosthetic group essential to its activity. This, the heme component consists of a porphyrin ring linked to a central iron atom (Figure 4a). The former consists of four pyrrole groups linked by methane bridges. In addition to four bonds with the four nitrogens of the porphyrin ring, heme iron possesses two additional linkages designated as fifth and sixth coordination positions (Figure 4b). Thus, a total of six interactions are possible; four in the plane of heme, and two projecting from it. The oxidation state of the iron is Fe^{+2} which can form a reversible complex with oxygen, but in the case of denatured hemoglobin and free heme, the state of the iron is Fe^{+3} (Antonini and Brunori, 1971). A folded globin chain surrounds each heme group. About 75% of each chain is folded in a right-handed helix. There are eight such helices designated A to H with AB, CD, EF, FG and GH corners in each chain. The hemoglobin molecule, composed of four such globin chains and their heme groups, is roughly spherical. Like enzymes, the outside of the globin chain is composed of both polar and nonpolar residues, while the interior part is made up almost entirely of nonpolar residues. The nonpolar environment is thought to protect the ferrous state (Fe^{+2}) of the heme iron from irreversible oxidation by excluding water (Antonini and Brunori, 1971). It should be noted, however, that two polar residues (the histidines alluded to earlier) are present in the interior. The

Figure 4 (a) Hemoglobin structure showing the four subunits being composed of hemes and globin chains

(b) The heme environment illustrating the heme group and the fifth and sixth coordination positions. 'W' represents any ligand which can bind to the heme group. (From Dickerson and Geis, 1983)





THE HEME ENVIRONMENT.

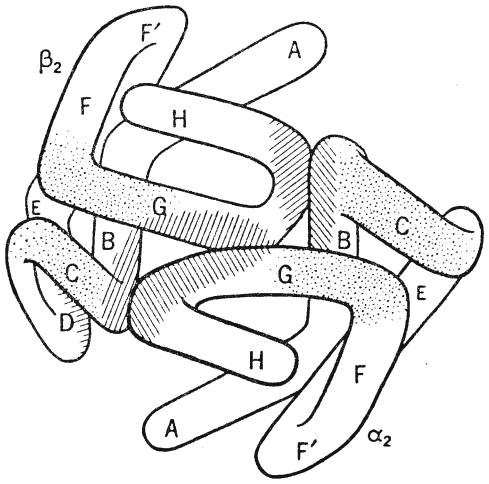
iron is bonded covalently to the imidazole of the proximal histidine 87 of the F helix which occupies the fifth coordination position. Actually, the iron atom is approximately 0.75°A out of the porphyrin ring plane toward the proximal histidine. The oxygen binding site is at the opposite side of this histidine; the sixth coordination position. Nearby is the distal histidine (58) of the E helix which is not bonded to the heme iron but directly to oxygen when the latter is inside the ligand pocket between the iron and the distal histidine. Another sixty atoms of the globin other than these histidines are in van der Waals contact with the porphyrin ring.

Although human hemoglobin α chains contain 141 residues, carp and desert sucker α chains have 142, and the trout, Salmo iridius, 144.

There are few contacts between α and α, or β and β subunits. The αβ contacts are of two types -- $\alpha_1\beta_1$ and $\alpha_1\beta_2$ ($\alpha_2\beta_2$ and $\alpha_2\beta_1$) and these are termed "packing" and "sliding" contacts respectively (Figure 5). The "packing" contacts involve the B, G and H helices and the GH corner of folded chains. Thus, subunit packing is unchanged when hemoglobin shifts from the deoxy- to the oxyconfiguration. The "sliding" contact which includes the C, G helices and the FG corner, however, undergoes changes during oxygen uptake and release; principally because of a change in the ligation state of the heme. Nearly one-third of these contacts involve formation of hydrogen bonds and saltbridges which are ruptured during ligand binding.

Perutz (1969, 1970) has investigated conformational changes in hemoglobin structure accompanying ligand binding. These can be summarized as follows. The iron which was 0.75°A out of the porphyrin ring plane is forced into the ring pushing the heme group closer to the F helix and histidine F8 to make room for the ligand. The histidine-iron bond is tilted away from an angle of 7°-8° with the heme plane to a perpendicular plane by ligand binding. Because the iron is bound to the proximal

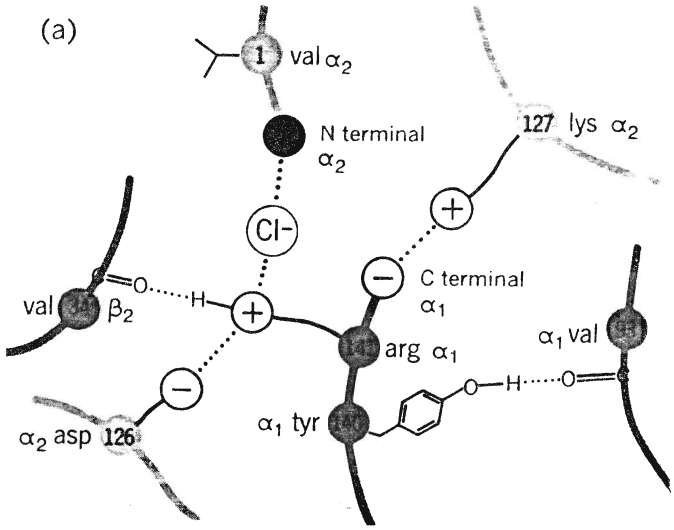
Figure 5 The $\alpha_2\beta_2$ dimer seen in a side view. Packing contacts (hatching) hold the dimer together and are unchanged when the molecule is oxygenated. The sliding contacts (stipple), however, undergo changes with oxygen uptake and release. (From Dickerson and Geis, 1983)


β_2
F′
A
F
H
E
G
B
B
C
C
G
E
D
H
F
A
F′
α_2

histidine, and the porphyrin ring is in contact with about sixty atoms of the globin chain, Perutz (1970) considers that these events are the primary triggers of the changes in tertiary and quaternary structure which take place during oxygen uptake and release. In the α chain, which has sufficient volume in the heme pocket for ligands, no large changes occur with oxygen binding. Consequently, the α units are likely to take up oxygen first. In the β chain, Valine β67 also shifts due to the primary triggers so that the distance between the porphyrin ring and the helix E increases and this facilitates subsequent ligand bonding. This movement is also one of the causes of changes in the hemoglobin configuration. When the molecule is deoxygenated, the space decreases in volume. This is a consequence of shifts in the F helices in both α and β chains towards the centre of the molecule and the H helices. The spaces between F and H helices are reduced and tyrosines, α 140 and β 145 are expelled from their F-H pockets. Expulsion of these tyrosines leads to displacement of C-terminal residues and the rupture of associated salt bridges and hydrogen bonds (Figure 6). More specifically, tyrosine α 140 pulls Arginine α 141 with it, breaking salt-bridges to Lysine 127 and Valine 1 of the opposite α subunit. This is accompanied by the release of Bohr protons. Hydrogen bonds with Aspartic acid 126 of the opposite α and Valine 34 of the opposite β subunit are also broken (Figure 6a). In each β chain tyrosine expulsion also leads to Histidine β146 displacement and the rupture of its salt-bridges with Aspartic acid β94 and Lysine 40 of the opposite α subunit. These events lead to the release of Bohr protons. The hydrogen bond between Tyrosine 145 and Valine β98 is also ruptured (Figure 6b).

The sliding contact between α and β subunits also changes with ligand binding (Figure 7). Histidine β97, which is located between the side chains of Proline α44 and Threonine α41 in the deoxy state is shifted past the threonine to a site between Threonine α41 and Threonine

Figure 6 Salt bridges and hydrogen bonds between other groups and the last two residues in (a) the α chains and (b) the β chains of deoxyhemoglobin. (From Dickerson and Geis, 1983)


(a)
1 val α_2
N terminal α_2
Cl-
127 lys α_2
C terminal α_1
val β_2
arg α_1
α_1 val
α_2 asp 126
α_1 tyr

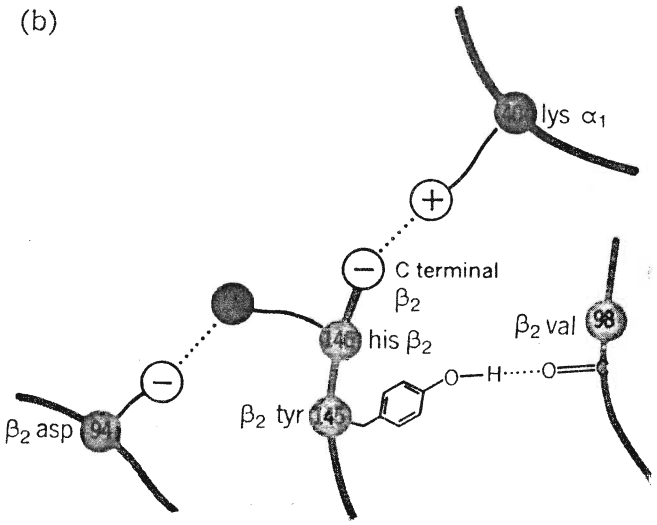
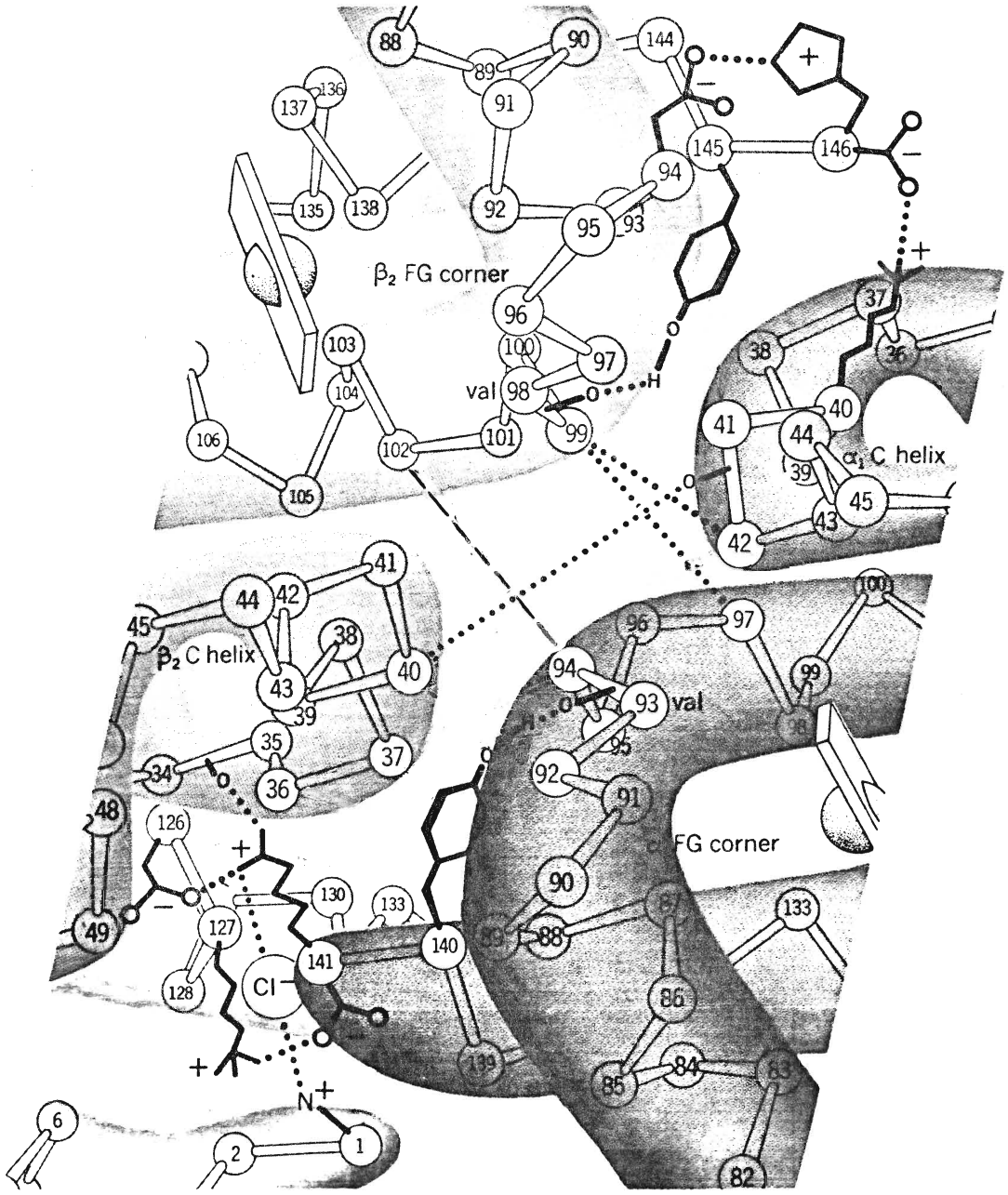

(b)
lys α_1
C terminal β_2
his β_2
β_2 val 98
β_2 asp 94
β_2 tyr

Figure 7 H-bonds and saltbridges at the $\alpha_1 \beta_2$ interface. Dotted lines represent bonds which are ruptured by ligand binding; dashed line indicates bond which exists in oxyhemoglobin. (Modified from Dickerson and Geis, 1983)


β2 FG corner
α1 C helix
β2 C helix
val
val
FG corner
Cl
N+

α38. The hydrogen bond between Aspartic acid β99 and Tyrosine α42 is also broken. Another hydrogen bond between Aspartic acid β99 and Asparagine α97 is replaced by one between Asparagine β102 and Aspartic acid α94. The rupturing of these saltbridges and hydrogen bonds, essential in stabilization of the deoxy or tense (T) form molecular configuration alters quaternary structure in favour of the relaxed (R) or oxyhemoglobin state.

With the foregoing structural concepts as background, consideration will next be given to the functional properties of hemoglobin, and the manner in which these can be adjusted during adaptation to altered environmental conditions. Particular consideration will be given to heme-heme interactions, the effects of carbondioxide, organic phosphates, inorganic constituents and temperature.

(1) Heme-heme interaction:

Oxygen equilibrium curves (OEC) summarize many of the important physiological properties of hemoglobins. Although the relationship between oxygen uptake by the monomer myoglobin and oxygen tension is a rectangular hyperbola, that of hemoglobin is characteristically sigmoidal in nature. Myoglobin thus binds oxygen readily, but requires a very low oxygen partial pressure for its release. Hemoglobin, on the other hand, loads and releases more readily. Weissblatch (1974), for example, notes that if myoglobin was the oxygen carrier, tissues would suffer from oxygen depletion. The sigmoidal character of the hemoglobin is largely attributable to heme-heme interactions. As would be infered from the foregoing section, oxygen binding to a heme group leads to conformational changes. Consequently, hemoglobin shifts from a tense (T) form to a relaxed (R) form; that is, it opens the other heme pockets so that ligands have easier access to iron. The first heme attached to the oxygen has

the lowest association constant (5-60 atm^{-1}), the exact value depending on pH, Cl^- level, CO_2 tension and the concentration of organic phosphates. The second and third hemes have association constants two to three times higher. The final heme has an association constant of 3000-6000 atm^{-1} (Dickerson and Geis, 1983).

Heme-heme interaction is commonly considered by reference to the Hill relationship;

$$\frac{Y}{1-Y} = Kp^n$$

where; Y = fractional saturation of oxygenated hemoglobin

1-Y = fractional saturation of deoxyhemoglobin

K = affinity constant

p = partial pressure of oxygen

n = empirical parameter (Hill's coefficient)

The 'n' is the tangent to the maximum slope of the OEC; i.e., between 10% and 90% oxygen saturation. The OEC has a slope of unity at very low (<10% P_{O_2}) and very high (>90% P_{O_2}) oxygen tensions. This suggests (Wyman, 1948) that heme units behave independently under very low and very high oxygen tensions. The slope, which becomes higher (i.e. >1) in the middle range of P_{O_2}, indicates heme-heme interaction. Although the 'n' value of human hemoglobin is in the range of 2.8-3.0, that of all trout hemoglobins is between 2.2 and 2.7 (Binotti et al, 1971). The shape of the OEC of mammalian hemoglobin is essentially independent of temperature and pH. Temperature invariance indicates that the heat of oxygenation is not a function of the degree of saturation. Similarly, pH invariance points to independence of the degree of saturation on the number of Bohr protons released (Wyman, 1979). By contrast, the n values and the positions of the OEC of most fish hemoglobins are functions of either pH or temperature, or both.

(2) The effect of carbondioxide:

The dependence of oxygen affinity on CO_2 was first reported by Bohr, C. (1904). In part this stems from reaction of CO_2 with primary amino groups of NH_2- termini to form carbamino groups (Henriques, 1929; Rossi-Bernardi and Roughton, 1967). This reaction can be described as:

$$CO_2 + PrNH_2 \rightleftharpoons PrNHCOOH \rightleftharpoons H^+ + PrNHCOO^-$$

This reaction leads to conversion of the neutral NH_2-termini of α chains to anions capable of forming saltbridges with Arginine α 141 (Figure 7). Consequently, carbamino formation favours the tense deoxy-hemoglobin configuration. The released H^+ also binds to Histidine β 146 forming a H-bond between Aspartic acid β94 and Histidine β146. This reaction can also occur at β NH_2-termini, but the release of H^+ may be balanced by a disruption of DPG binding (see below), and because of this promotes the relaxed or oxyconfiguration (Kilmartin and Rossi-Bernardi, 1969; Dickerson and Geis, 1983). Farmer (1979) has described specific CO_2 effects on the hemoglobins of a number of South American fishes, lungfish, Lepidosiren paradoxa; catfish, Brachyplatystoma sp. and hoplo catfish, Hoplosternum littorale. Given comparable CO_2 tensions, the effect was roughly the same for all hemoglobins tested. The single exception involved the Amazonian catfish, Brachyplatystoma spp. In this instance, the effect of CO_2 was approximately twice as great as that for other species, and equal to that found in human hemoglobin. This similarity suggests that the NH_2-termini of the globin chains in this fish hemoglobin behave as do those of human hemoglobin. The lesser CO_2 effect on other hemoglobins such as those of carp and trout, may stem from blocking of α chain NH_2-termini. In the α chains of carp and trout hemoglobins, the N-terminial amino acid residue, serine is acetylated preventing the binding of CO_2 to the chanins (Hilse and Braunitzer, 1968; Bossa et al, 1976). However, in the β

chains of carp and trout hemoglobins, the N-terminal residues, Valines, are free as in human hemoglobin. Consequently, they can react with CO_2 to form carbamino compounds.

The second, and more important CO_2 effect is termed to the Bohr effect; a decrease in oxygen affinity with decrease with pH. In aqueous solution, CO_2 is hydrated to carbonic acid which, in turn, dissociates into H^+ and HCO_3^- ions. Within red cells the reaction is catalyzed by carbonic-anhydrase; an enzyme notable for its very high turnover number.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

This effect relates to the action of carbonic acid as a proton donor, rather than to CO_2 per se. CO_2 enters the red cells, and carbonic anhydrase generates HCO_3^- and H^+. Since the red cell membrane is more permeable to HCO_3^- than H^+, intracellular pH decreases and H^+ (Bohr protons) combines with hemoglobin, enhancing oxygen unloading through facilitation of the tense, deoxyhemoglobin configuration. Although the original Bohr effect was described only in terms of CO_2, the term is now used both for the effects of CO_2 and pH. Increased red cell $[H^+]$ within the range of pH 6 and 10 favors the tense configuration through protonation of Histidine β 146, the amino termini of the α chains, Histidine α 122 and Histidine β 143 and the strengthening of saltbridges and hydrogen bonds (Dickerson and Geis, 1983). Hence, it can be said that the Bohr effect is the result of a competition between the bindings of oxygen and H^+ to hemoglobin.

$$HbH^+_n + O_2 \rightleftharpoons HbO_2 + nH^+$$

Bohr effect can be described in equation form,

$$(\emptyset) \text{ Bohr factors} = \frac{\Delta \log P50}{\Delta pH}$$

pH is thus one of the most important factors influencing the position of the OEC. For example, in rainbow trout, the OEC is shifted

to the right below pH 7.3, decreasing oxygen affinity (Brunori, 1975). The effect of pH on the shape of the OEC; i.e., change in 'n' value, has also been examined in fish hemoglobins (Root, 1931; Manwell, 1960). In the case of rainbow trout, HB IV (Brunori, 1975), the n value changes from a maximum of approximately 2.0 at pH 7.5 to less than 1 at pH values below 6.5 and above 8.0 (Weber et al, 1976).

(3) The Root effect:

Root (1931) observed that fish blood showed a marked loss of oxygen capacity at high CO_2 levels; the so-called Root effect. In the Bohr effect, CO_2 and H^+ shift the OEC to the right and, because of this, hemoglobin unloads oxygen. In the instance of the Root effect, oxygen loading is actually reduced as CO_2 and $[H^+]$ increases. As mentioned earlier, the shape of the curve is determined by the value for n. If n is less than unity (for example, in rainbow trout HB IV as noted above) it shows negative heme-heme interactions (Manwell, 1960). This diminution in oxygen carrying capacity at relatively high and low pH values is thus quite distinct from the Bohr effect on oxygen affinity (Manwell, 1960).

(4) The effect of organic compounds:

The red cells of many vertebrate species generate organophosphates of various types, and these are normally retained in the cells. Organophosphate binding to hemoglobin, with consequent effects upon oxygen affinity was initially reported by Chanutin and colleagues (Sugita and Chanutin, 1963; Chanutin and Curnish, 1964; Ludewing and Chanutin, 1964). The anucleated erythrocytes of mammalian species typically form 2,3-DPG, but contain ATP, GTP and other compounds in lesser concentrations. In the nucleated red cells of non-mammalian vertebrates, a wide range of such materials are found, with ATP and GTP predominant in fish erythrocytes (Bartlett, 1970; Gillen and Riggs, 1971; Powers and Edmundson, 1972a,

Wood and Johansen, 1972; Geohegan and Poluhowich, 1974). In terms of their effects on mammalian hemoglobins: 2-3DPG > GTP > ATP > ADP > AMP > pyrophosphate > inorganic phosphates (Bunn et al, 1971). Organophosphate modulation of hemoglobin-oxygen affinity has been studied most extensively with respect to 2,3 - DPG and ATP. The influence of organophosphate compounds on affinity is formally identical to that of protons, since all involve heterotropic allosteric interactions between oxygen and phosphate binding sites. The overall reaction is as below:

$$HbO_2 + NTP \rightleftharpoons Hb.NTP + O_2$$

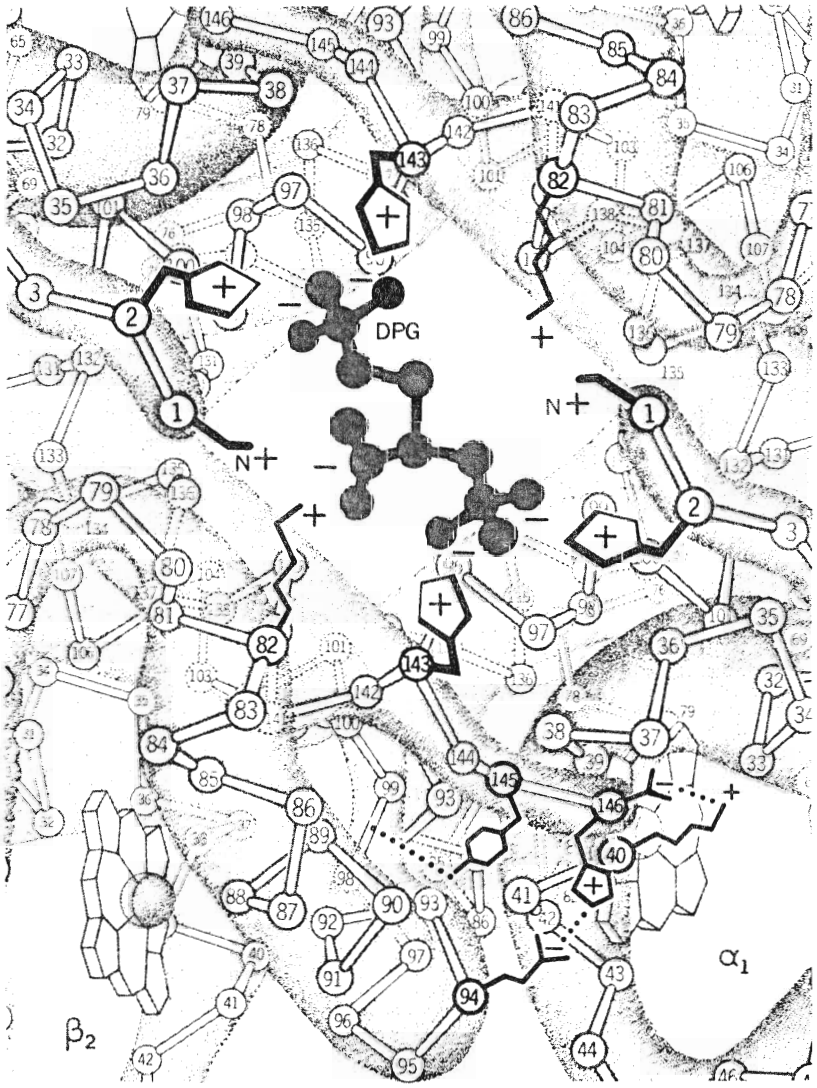
In human hemoglobin, one molecule of 2,3- DPG binds between the ends of the β chains of deoxyhemoglobin (Figure 8). The five negative charges of organophosphate interact with the positive charges on the side chains of Histidine β 2, one of the 2 Lysines β 82, Histidine β 143 and Valine β1 (Arnone, 1972). In the oxygenated state, however, this 2,3- DPG pocket becomes smaller due to the movement of β chains. Consequently, the DPG molecule is, in a sense, "squeezed" out of its pocket. It is believed that this site is responsible for organic phosphate binding in fish hemoglobins as well, because the homologous residues, Valine β1, Lysine β82, Arganine β143 and Glutamic acid β2, are found here in most fish hemoglobins (Powers and Edmundson, 1972b).

In addition to allosteric effects, these compounds have another influence on oxygen affinity. Since the red cell membrane is impermeable to organophosphates, they also affect the Donnan distribution of protons (Wood and Johansen, 1973a). Decreases in ATP concentration, for example, allow more H^+ to move out of the erythrocyte. Because of this intraerythrocytic pH increases, and the anticipated effect of reduced H^+ concentration is seen; i.e., affinity increases.

Because of their common binding sites on β chains, CO_2 and

Figure 8 Amino acid side chains around the DPG (ATP)-binding site.

(From Dickerson and Geis, 1983)


DPG
N+
N+
β_2
α_1

organophosphate molecules compete with each other. This relationship is obvious in many experiments. For example, in carp hemoglobin, organophosphates mask CO_2 effects below pH 8.3. At higher pH values, the CO_2 effect is more pronounced, even in the presence of organophosphates. This is thought to reflect increased carbamate formation as competition with organophosphate decreases (Weber and Lykkeboe, 1978).

The decrease in organophosphate effects at high pH levels was studied in some detail by Powers and Edmundson (1972a,b), Gillen and Riggs (1972), Mied and Powers (1978), and Greaney and Powers, 1978. As mentioned earlier, phosphates can bind only to deoxyhemoglobin molecules which are highly protonated at their β chain NH_2- termini. At high pH, H^+ ions are scarce and β-NH_2 termini are only partially protonated. Consequently, hemoglobin-phosphate affinity is reduced as pH increases. This occurrence has been tested in fishes such as carp (Gillen and Riggs, 1972; Weber and Lykkeboe, 1978) and rainbow trout (Weber et al, 1976b).

(5) The Effects of inorganic ions:

Both monovalent and divalent inorganic ions are effective modulators of hemoglobin-oxygen affinity in vertebrates. The divalent cations, Ca^{+2} and Mg^{+2} form complexes with nucleoside triphosphates (NTP) denying interaction with hemoglobin. Thus, by increasing red cell Mg^{+2} the effective level of organophosphate concentrations that able to bind to NH_2-termini of the β chains can be reduced, and vice versa. Because nucleoside triphosphates such as ATP, GTP are of paramount importance in fishes, adjustments of this kind are not uncommon (Weber and Lykkeboe, 1978; Houston and Koss, 1984b). The result of an increase in Mg^{+2}, NTP being constant, is to shift the OEC left. Deoxyhemoglobin and Mg^{+2} are competitors, binding reciprocally with organic phosphates (Bunn et al, 1971), and their relative affinities are: HbDPG > HbATP > MgATP > MgDPG.

An interesting outcome of this in mammals is that ATP is rarely involved in affinity modulation despite its abundance in erythrocytes ($[ATP] \simeq 0.2 \times [2, 3 - DPG]$). Compared to Ca^{+2}, Mg^{+2} is more effective in modulation. Bunn et al (1971) have estimated that approximately two-thirds of the Mg^{+2} present in the deoxygenated mammalian red cell is ATP-complexed.

Other anions influence the relationship between the relaxed and tense hemoglobin configurations. Notable among these is chloride. Increases in Cl^- level act functionally as do H^+, and organophosphates to decrease oxygen affinity in the absence of DPG or ATP (Benesch and Benesch, 1967). In addition to sites between the two β chains, Cl^- binding sites are also located at the NH_2-termini of α chains (Figures 6a and 7). Here, Cl^- assists protonation, and forms a saltbridge between the α NH_2 terminal residue and Arginine 141 of the opposite α chain.

Like Ca^{+2} and Mg^{+2} the monovalent cations, Na^+ and K^+, can bind weakly to organophosphates (O'Sullivan and Perrin, 1964; Perrin and Sharma, 1966), and influence interactions between organophosphates and divalent cations to some extent. Such monovalent cation-organophosphate complexes are, however, characterized by low stability constants and they do not greatly affect oxygen-hemoglobin affinity. In the carp, Na^+ slightly increases the oxygen affinity of stripped hemoglobin, indicating some facilitation of interactions between Na^+ and oxygen binding sites (Weber and Lykkeboe, 1978). Rossi-Fanelli et al (1961) and Bunn et al (1971), however, showed that at equivalent concentrations, K^+ reduces oxygen-hemoglobin affinity more than did Na^+. The effects of Na^+ and K^+ are, however, small in comparison to those of other modulators.

(5) The effect of temperature:

Temperature effects on oxygen affinity were first reported by

Barcroft and King (1901) who noted that increases in temperature reduced affinity. Because the binding of oxygen to hemoglobin is commonly an exothermic reaction which follows Le Chatelier's principle, this is not surprising. However, there are some exceptional cases among teleosts. For example, tuna hemoglobin is not sensitive to temperature, (Weber et al, 1976). Increasing temperatures are associated with increased metabolic oxygen demand and reductions in pH (Eddy, 1971). If the hemoglobins of ectotherms have sensitivity to both temperature and pH, this dual effect can easily shift the OEC to the right, and drastically change the affinity.

Johansen and Lenfant (1972) hypothesized that lowering the temperature dependence of hemoglobin might be an appropriate adaptation to large fluctuations in environmental temperature, citing as examples the Australian and African lungfish, and the bottom-dwelling skates. Powers, et al (1979), however, pointed out many exceptions to this generalization, noting that ". . . the temperate species in our study have hemoglobins with thermal sensitivity similar to those of their tropical counterparts. . ." Moreover, they also noted that the euryhaline killifish, Fundulus heteroclitus, whose habitat exhibits both annual and daily thermal gradients possesses hemoglobins as thermally sensitive as those of fish from constant thermal environments. They, therefore, concluded that ". . . a generalized theory regarding hemoglobins, habitat and thermal stability does not appear to be justified. . ."

III. Multiple Hemoglobin Components:

In contrast to the mammals and birds, fish, amphibians and reptiles typically exhibit a multiplicity of hemoglobins (Gratzer and Allison, 1960). As all are ectotherms, Riggs (1970) has suggested that possession of multiple hemoglobins may confer adaptive advantages. Riggs (1970)

also discussed the occurrence of multiple hemoglobin components in poikilotherms and pointed out the possible causes. Among the more important of these are the following:

(1) Reaction of NH_2-terminal group of the polypeptide chain:

Binding of an acetyl or other blocking group to the NH_2-termini of globin chains causes changes in the net charge on the hemoglobin molecule. NH_2-termini of the α chains of all fish hemoglobins tested so far including trout's are acetylated. If this reaction is incomplete, multiplicity of hemoglobin components will occur.

(2) Allosteric Effectors:

The binding of DPG, ATP and inositol polyphosphates to deoxygenated hemoglobin produces an electrophoretically distinct component (Chanutin and Curnish, 1964, 1967; Benesch and Benesch, 1967, 1969).

(3) Environmental conditions:

Because of their dependence on the metabolism, organophosphate levels in red cells often vary with environmental conditions. Through the levels of allosteric modulators, it can be assumed that environmental conditions also cause multiple Hb components.

(4) Mutation:

The wide-spread occurrence of multiple components with different amino acid compositions and functions, indicating extensive gene duplication followed by mutation is a major cause of multiplicity.

Riggs (1979) reviewed a functional classification of fish hemolysates described by Weber et al (1976b) as follows.

Class I hemolysates contain one to several hemoglobin components, all of which are sensitive to pH and all have similar but not identical functional properties. This class includes the hemolysates of carp, the Rio Grande cichlid (Cichlasoma cyanoguttatum), plaice, flounder and the bowfin, Amia. Class II hemolysates contain multiple components

which are different in functional properties. Generally, the electrophoretically anodal components are like those in Class I, but the cathodal components lack the normal pH and temperature sensitivities of ligand binding. The hemolysates of trout, eel, salmon belong to this class. Class III hemolysates such as that of tuna, are pH- but not temperature-sensitive in ligand binding.

It is assumed that hemoglobin isomorphs with different physiological properties participate in hematological adaptation. For example, the proportions of certain hemoglobin polymorphs change with thermal acclimation in trout and goldfish (Houston and Cyr, 1974; Weber et al, 1976c). Weber et al (1976c) suggest that hemoglobins which are insensitive to temperature may stabilize the oxygen affinity when the temperature varies.

IV. Adaptive Hematological Response - Hypoxia

As noted previously, oxygen availability is a critical factor for aquatic organisms, and especially for those living in habitats in which they are exposed to hypoxia. To some extent, metabolic shifts can compensate for this. Under hypoxic circumstances, blood lactate levels in fishes are elevated significantly as a result of increased anaerobic metabolism. Holeton and Randall (1967), for example, demonstrated that in rainbow trout blood lactate increased by a factor of two to three following exposure to hypoxia. Similar results were reported by Greaney and Powers (1978) for Fundulus heteroclitus and by Black (1955) in several salmonid species. Both arterial and venous P_{CO_2} levels also increases under hypoxic conditions. For example, in rainbow trout, P_{CO_2} in arterial and in venous blood were 1-1.5 and 2.5 mmHg respectively under normoxic conditions, but increased to 3.5-4 and 4.5-5.00 mmHg at hypoxia (Holeton and Randall, 1967). The latter

values are close to the maximum levels seen in aquatic animals. Consequently, blood pH decreases. In the study cited, for instance, pH under normoxic conditions was 7.7 but fell to 7.4 during hypoxia (Holeton and Randall, 1967). Decreases in blood pH, of course, decrease oxygen-carrying capacity (Root effect). There are also many reports indicating a decrease in the arterial oxygen tension and in the difference between the arterial and venous oxygen tensions in fish (Holeton and Randall, 1967 ; Wood and Johansen, 1973b; Eddy, 1971). Because of these effects, if there were no adaptations in the blood oxygen binding properties, oxygen-requiring tissues would seriously suffer from oxygen deficiency. Although acidosis is an adaptation in some hypoxic teleosts such as rainbow trout occupying well-oxygenated waters (Soivio et al, 1980), hypoxic carp (Weber and Lykkeboe, 1978) and killifish (Greaney and Powers, 1978) did not show any changes in blood pH.

Soivio et al (1980) have concluded that no variation in blood pH of fishes, for example, carp and killifish which occupy less-oxygenated waters, is well suited to withstand low environmental oxygen level in this kind of habitat.

Powers (1980) and Weber (1982) generalized two types of responses found under such circumstances; an immediate cardiovascular-ventilatory response, followed by acclimatory hematological response. The primary concern of this study is with hematological aspects of response and, in particular, those associated with oxygen-carrying-capacity and hemoglobin-oxygen affinity. As noted initially, the former, with cardiac output, establish the maximum rates at which oxygen can be delivered to tissues. It is the latter, however, which govern the extent to which this potential can be realized. Studies on acclimatory changes in oxygen carrying capacity have generally involved measurement of all, or some of the

primary hematological indices (Hemoglobin, hematocrit, red cell number) and parameters derived from these (mean erythrocytic volume, mean erythrocytic hemoglobin, mean erythrocytic hemoglobin content). The latter are defined as:

Mean erythrocytic volume = Hct (10) / RBC ($10^6/mm^3$)

Mean erythrocytic hemoglobin = Hb (100) / Hct

Mean erythrocytic hemoglobin content = Hb (10) / RBC (10^6 / mm^3)

where: Hct = hematocrit (%)

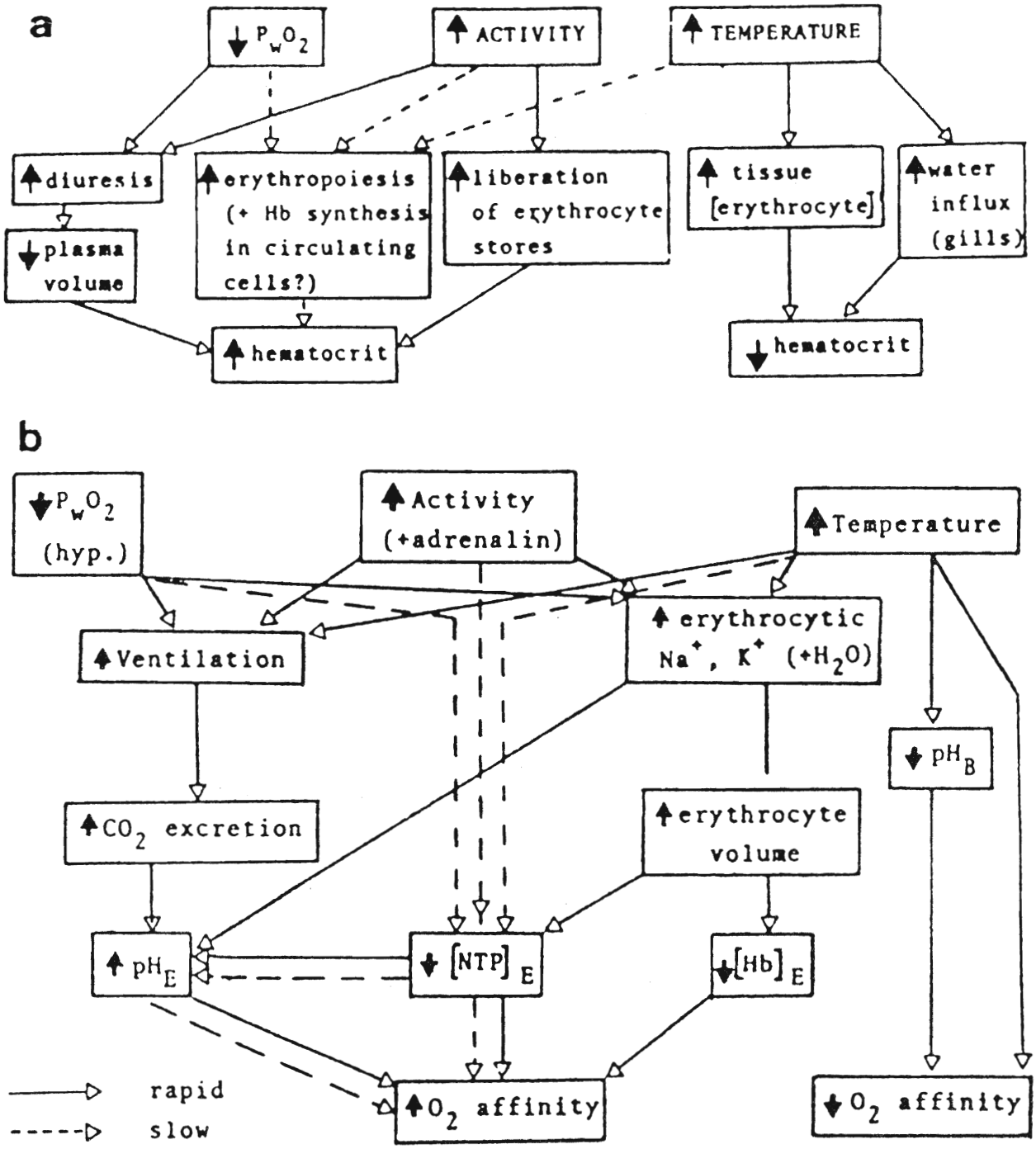
RBC X $10^{-6}/mm^3$ = erythrocyte number

Hb = blood hemoglobin content ($g.dl^{-1}$)

The most commonly measured and most readily determined primary index is hematocrit (Figure 9). Increases in hematocrit which may be indicative of increased oxygen-carrying capacity may stem from the liberation of stored erythrocytes from the spleen (Johansen and Hanson, 1967; Lane, 1979) or, in the longer term, from increased erythropoiesis. However, they may also result from increased diuresis (Wood and Randall, 1973; Swift and Lloyd, 1974) and a decline in plasma volume, from erythrocyte swelling (Black and Irving, 1938; Soivio and Nikinmaa, 1981; Nikinmaa and Soivio, 1982) or from a shift of extracellular fluids into the cellular compartment (Cameron, 1970). The latter factors also influences the other primary indices as well.

Furthermore, specimen size may influence response. Smeda and Houston (1979), for example, reported marked hematological responses in smaller carp, and no obvious response in larger animals. They suggest that discrepancies found in the literature may stem, in part at least, from this. Beyond this, of course, a number of factors may affect capacity to respond. Notable among the latter is nutrition. Although increased blood hemoglobin content is regarded as a critical form of adaptation, it does not seem to be a consistent feature of response (Weber, 1982; Nikinmaa and Soivio, 1982). For example, in

Figure 9 Effects of water O_2 tension ($P_{w\ O_2}$), activity and temperature on (a) O_2 carrying capacity and (b) O_2 affinity, as observed in blood of various fish. (From Weber, R. E., 1982)


a
↓ P_wO_2
↑ ACTIVITY
↑ TEMPERATURE
↑ diuresis
↑ erythropoiesis (+ Hb synthesis in circulating cells?)
↑ liberation of erythrocyte stores
↑ tissue [erythrocyte]
↑ water influx (gills)
↓ plasma volume
↑ hematocrit
↓ hematocrit
b
↓ P_wO_2 (hyp.)
↑ Activity (+adrenalin)
↑ Temperature
↑ Ventilation
↑ erythrocytic Na^+, K^+ ($+H_2O$)
↓ pH_B
↑ CO_2 excretion
↑ erythrocyte volume
↑ pH_E
↓ $[NTP]_E$
↓ $[Hb]_E$
↑ O_2 affinity
↓ O_2 affinity
rapid
slow

carp (Weber and Lykkeboe, 1978) and rainbow trout (Soivio and Nikinmaa, 1981; Nikinmaa and Soivio, 1982) hemoglobin content did not change significantly under hypoxic conditions. On the other hand, Soivio et al (1980) reported a 25% increase in the oxygen-carrying capacity of rainbow trout within 6 hours of exposure to hypoxia. This resulted from elevation in both hematocrit and hemoglobin content.

Hemoglobin-oxygen affinity increases under hypoxic circumstances; partly as a result of erythrocyte swelling (Weber et al, 1976a; Soivio and Nikinmaa, 1981) (Figure 9). Such volume increases may be under hormonal control. Kregenow (1973) reported that noradrenaline induced swelling in duck erythrocytes. Injection of adrenaline prompts a similar response in rainbow trout at 14^{o}C (Nikinmaa, 1981). Rudolph and Greengard (1980) suggested that swelling under isosmotic condition is under adrenergic control, and caused by inward cotransport of Na^{+} and K^{+}, followed by water. Black and Irving (1938), who first described this phenomenon, thought that it might be a result of increases in blood lactic acid levels. Later Irving et al (1941) and Benditt et al (1941) hypothesized that changes in P_{CO_2} was the initiating factor. In flounder, Platichthys flesus, (Fugelli, 1967) and rainbow trout (Soivio and Nyholm, 1973) such swelling is reversible: when exposed to reduced PO_2 erythrocytes swell, but then shrink when oxygen tensions are increased.

Erythrocyte swelling constitutes an interesting aspect of response because of the effects of volume changes on oxygen affinity (Weber et al, 1976a). Reductions in erythrocytic hemoglobin and organophosphate concentrations are combined with increased cell volume. This reduces organophosphate-hemoglobin interaction, and is coupled with increases in pH as a result of changes in the Donnan distribution of H^{+} (Wood and Johansen, 1973a). Consequently, oxygen affinity is raised. In carp acclimated to hypoxia, for example, P50 decreases from 7.0 to 3.0 mmHg (Weber and Lykkeboe, 1978). Similar results have also been reported for

eel, Anguilla anguilla (Wood and Johansen, 1973a; Weber et al, 1975), the benthic flounder, Pleuronectes platessa (Wood et al, 1975) and rainbow trout (Soivio et al, 1980; Soivio and Nikinmaa, 1981).

Dilution of concentration of modulatory solutes by volume changes is an essentially passive phenomenon. Organophosphate levels can also be adjusted in a more active fashion by changes in oxidative phosphorylation. This process is the primary source of most organophosphates in nucleated cells and modulator synthesis is oxygen-dependent. It is not surprising, therefore, that organophosphate levels in red cells decrease during hypoxic stress. The resulting decrease in [ATP] : [Hb] ratio then leads to increased oxygen affinity, enhancing branchial oxygen uptake and partially, at least, compensating for the initiating stress (Greaney and Powers, 1978).

The question of hemoglobin system organization in relation to oxygen availability, one of the foci of this study, has received relatively little attention. Indeed, the biological significance of hemoglobin heterogeneities has not been clearly demonstrated although the hypothesis has been advanced that such heterogeneity may represent adaptation to variable environments (Sullivan, 1977). Several studies have demonstrated changes in hemoglobin isomorph abundancies in relation to the thermoacclimatory process (Houston and Cyr, 1974; Houston et al, 1976; Houston and Rupert, 1976). Seasonal variations in the relative proportion and number of isomorphs in carp and yellow fish, Barbus holubi have also been reported (Fourie and Van Vuren, 1976). The effect of hypoxia has not been considered in detail as yet. However, Wood and Johansen (1972) in studies upon the eel, Weber and Lykkeboe (1978) on carp and Giles and Vanstone (1976) on coho salmon, Oncorhynchus kisutch, could detect little change in hemoglobin component abundancies in relation to hypoxia. In part, at least, this may reflect the relatively insensitive

electrophoretic procedures employed.

To have potential adaptive value, the components of multiple hemoglobin system must satisfy two criteria. First, they must exhibit functional differences. Second, they must exhibit adaptively appropriate changes in abundancy either through deaggregation-reaggregation phenomena or as a consequence of differential synthesis. Among the hemoglobin isomorphs of eels, the cathodic HbI has lower P50 and higher n values than do anodic components (Weber et al, 1975. Not only eel HbI is more sensitive to organophosphates than the anodic fractions, it also exhibits a reverse Bohr effect (increase in P50 with increasing pH). Because of these qualities, the P50 of HbI is strongly reduced; i.e., its oxygen affinity increased during hypoxia as a result of the decreased phosphate contents (Weber et al, 1975). Thus, Weber et al (1975) concluded that HbI is mainly responsible for the adaptation of blood oxygen affinity to ambient oxygen tensions. Although the authors did not report whether the actual contents of these isomorphs varied with hypoxia or not, no apparent influence on their relative concentrations was observed. Brunori et al (1979) have also examined Amazonian catfish, Pterygoplichthys parodalis, a species usually found in hypoxic areas, in this respect. The major hemoglobin fraction, approximately 50% of total hemoglobin, has a comparatively small Bohr factor and exhibits ATP-dependent variations in affinity. The other group comprising Components II, III and IV are characterized by very large Bohr factors and oxygen affinity independent of ATP.

V. Adaptive Hematological Responses - Temperature

Several studies (De Wilde and Houston, 1967; Houston and De Wilde, 1969; Cameron, 1970; Houston and Cyr, 1974) have reported thermo-acclimatory increases in hemoglobin content in brook and rainbow trout,

goldfish, pinfish, Lagodon spp. and striped mullet, Mugil cephalus. This is usually associated with increase in the number of red cells, reduction in mean erythrocytic volume and minor increases in cellular hemoglobin content (Houston, 1980). However, as previously noted, responses of this type are notable for inconsistencies in magnitude and occurrence. For example, although two studies (De Wilde and Houston, 1967; Houston and Cyr, 1974) on the rainbow trout demonstrated elevation in overall hemoglobin content and red cell numbers, moderate reductions in cell volume and minor increases in mean erythrocytic hemoglobin content, another study by Houston and Smeda (1979) showed statistically insignificant changes of these parameters.

Decreases in hemoglobin-oxygen affinity, which would facilitate oxygen release to tissues, are a common result of thermal acclimation (Figure 9). These have been demonstrated in a number of species including the lungfish, Lepidosiren paradoxa, hoplo catfish, Hoplerythrinus unitaeniatus, catfish, Synbranchus marmoratus (Powers et al, 1979) and Australian blackfish, Gadopsis marmoratus (Dobson and Baldwin, 1982). In part, at least, these decreases in P50 can be ascribed to changes in overall enthalpy (ΔH obs). The latter includes intrinsic heat of oxygen binding (ΔHo), heat of solution of oxygen (ΔH sol)aand heat of protonation and of the blood buffer system (ΔH_i and ΔHb) (Wyman, 1964; Weber, 1982).

$$\Delta Hobs = \frac{2.303\ R\ \Delta\ \log P50}{\Delta 1/T}$$

Reduction in ΔH is thought to be an important adaptation in some species of fish living in thermally-fluctuating environments since it leads to a lowering in temperature sensitivity. This has been reported in rainbow trout (Brunori, 1975), desert sucker (Powers, 1980) and the blue fin tuna, Thunnus thynnus (Rossi-Fanelli and Antonini, 1960). Hochachka and Somero (1973) suggest that adaptations of this kind in

species such as tuna are associated with maintenance of thermal gradients between the peripheral and core body regions. If tuna hemoglobin had a "normal" ΔH, cool, peripheral blood entering the warmer, deep muscle mass might well unload oxygen very quickly, prompting formation of gas emboli. Generally, relatively temperature-independent hemoglobins in polymorphic system are accompanied by other components displaying more thermal sensitivity (Powers, 1980). For example, in rainbow trout the Brunori fraction I (Brunori, 1975) exhibits a ΔH of -3 to -4; that of fraction IV is -15 to -16 Kcal $mole^{-1}$. As noted earlier, however, evidence supporting the concept of evolutionary development of thermally less sensitive hemoglobin components in fishes living in fluctuating thermal environments is not strong (Powers, 1980) although there are exceptions, acclimation to higher temperatures is often associated with decreases in hemoglobin-oxygen affinity. For example, the killifish of daily temperature fluctuating environment has hemoglobin components with high ΔH, -15 to -16 Kcal.$mole^{-1}$.

Furthermore, some teleosts such as the bowfin, Amia spp. (Johansen and Lenfant, 1972), the bullhead, Ictalurus nebulosus (Grigg, 1969) and killifish (Powers, 1980) display the opposite response in that increases in oxygen affinity are seen with increasing temperature. The hemoglobin of Ictalurus has a very high oxygen affinity and low Bohr effect, and these properties are considered as adaptations to live in stagnant waters of low oxygen content; i.e., they permit maximum usage of such oxygen as is available (Haws and Goodnight, 1962).

As described earlier intraerythrocytic pH, organophosphate levels and intraerythrocytic ion concentrations contribute to adjustments in oxygen-hemoglobin affinity. In Australian blackfish, both total NTP level and [NTP]:[Hb] are significantly increased while pH is decreased upon exposure to a temperature increase from 10° to $20^{\circ}C$ (Dobson and

Baldwin, 1980). These two factors seemed to be the major modulators in regulating the oxygen affinity in this species. A decline in [ATP]:[Hb], however, was reported in catostomid species (Powers, 1974) and killifish (Powers, 1980) - increasing hemoglobin-oxygen affinity - following acclimation to higher temperatures. Interestingly, Weber et al (1976b) reported no significant charges in NTP and [NTP]: [Hb] in rainbow trout acclimated at 5°, 15° and 22°C indicating no effect of acclimatory temperature. Houston and Koss (1984b) reported recently that at near-lethal higher temperatures (24 - 26°C) NTP level increased. Hemoglobin drops sharply, $[Mg^{+2}]$: [ATP] declines and [NTP] : [Hb] increases favouring an increase in organophosphate influence.

Thermal acclimation also leads to changes in cellular levels of modulatory inorganic ion as well. Several studies on the variations in ionic compositions of fish erythrocytes have been carried out (Houston and Mearow, 1979; Houston and Smeda, 1979; Koss and Houston, 1981; Houston and Koss, 1984a,b). Houston (1980) reviewed ionic changes in rainbow trout and carp under thermal stress. Red cell K^{+} levels increase significantly, but Na^{+} is reduced equivalently by acclimation of rainbow trout to high temperature. Chloride level is moderately elevated, with the converse being true of Mg^{+2} and Ca^{+2}. In carp, K^{+} concentration is similar to that found in trout, but changes are insignificant. Compared to trout, Cl^{-} level is elevated sharply. Nondetectable amount of Na^{+} in the erythrocytes of carp held at 2°C rises five times between 16° and 30°C. Contrast to Ca^{+2} which erythrocytic concentration is relatively thermostable, Mg^{+2} level is reduced sharply.

Ion : hemoglobin ratios were also described by Houston (1980). Those of Cl^{-}, Mg^{+2} and Ca^{+2} to hemoglobin in rainbow trout are thermally stable. However, K : Hb rises sharply at higher temperatures. [K] :[Hb] does not vary significantly in carp although both $[Cl^{-}]$[Hb] and $[Na^{+}]$:[Hb]

are markedly elevated.

The multiple hemoglobin system of teleosts are noteworthy for both quantitative and qualitative changes following thermal acclimation (Houston and Cyr, 1974; Houston et al, 1976; Houston, 1980). The goldfish represents unique example of the latter response. Animals held at 30°C exhibit three hemoglobin fractions of which one, 'G1' component is absent at 5°C (Falkner and Houston, 1966; Houston and Cyr, 1974; Houston et al, 1976). The most abundant (70-82%) fraction, G2 declines significantly, while G3 (18-24%) increases at 30°C (Houston and Cyr, 1974).

This qualitative response found in goldfish is quite distinct from that in the related carp. This species consistently exhibits three isomorphs between 5° and 30°C (Houston et al, 1976). All components are altered significantly; two minor components (C_2, C_3) increase in abundance at higher temperatures while the principal isomorph, (C_1) declines. In rainbow trout, 9 well-defined fractions are consistently observed at all temperatures (Houston and Cyr, 1974). Seven, including the two major components changed significantly in their actual concentrations.

MATERIALS AND METHODS

(1) Experimental stock and holding method:

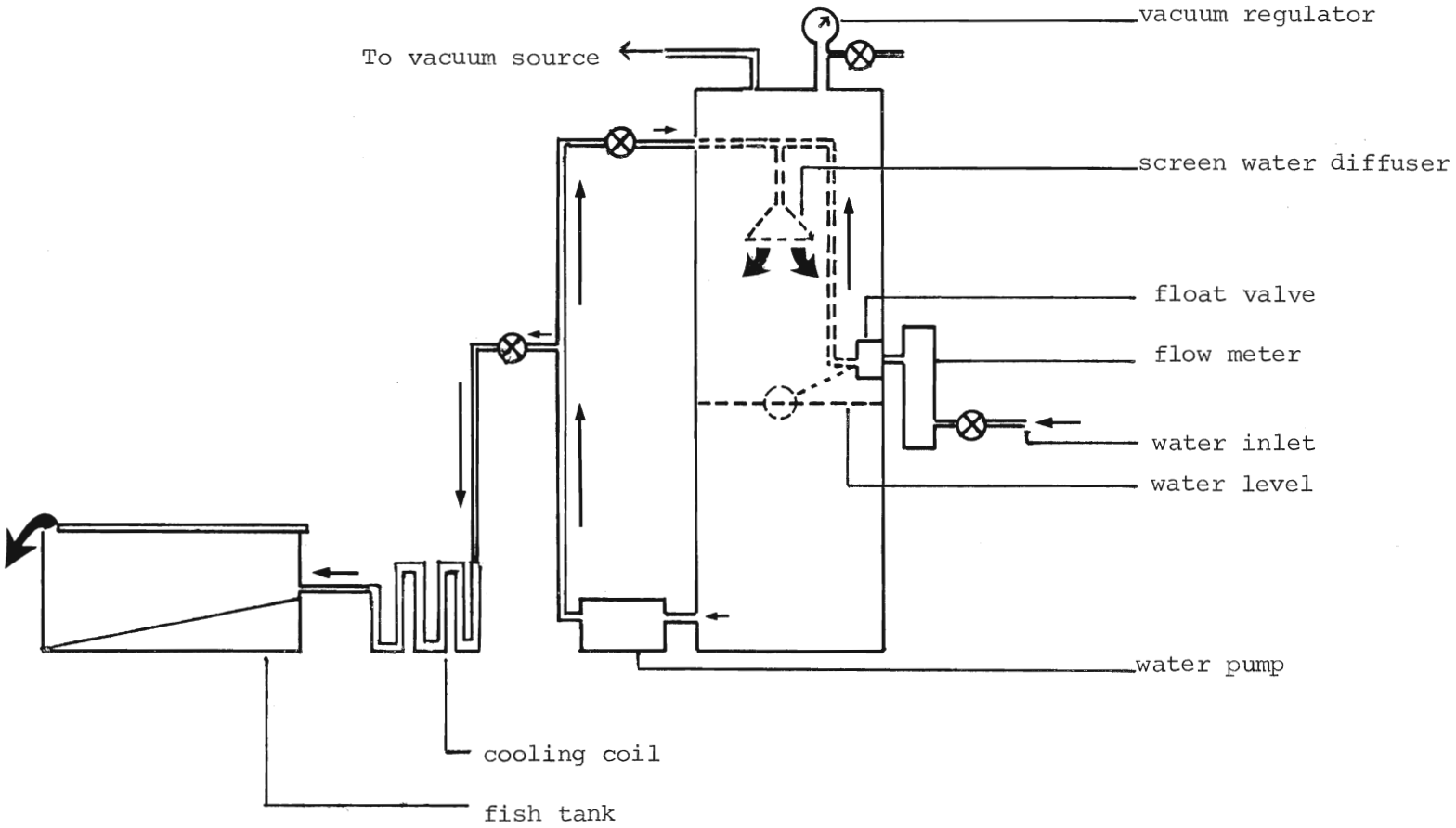
Rainbow trout having a mean weight of 73.0 g (39.5 - 108.9 g) were obtained from a commercial supplier, Goosen's Trout Farm, Otterville, Ontario. They were inspected upon arrival and divided into two groups, both of which were held in recirculating, double-walled fibreglass tanks (LS-700, Frigid Units Inc., Toledo, Ohio). Water in these tanks cycled completely every1-½ minutes. Consequently, there was little evidence of spatial difference in dissolved oxygen levels and temperature. Each tank was equipped with an activated charcoal Cul-Brook water softening - dechlorination - filtration system (Culligan International Comp., Mississauga, Ontario) and a cooling system (BHL - 1076 coolers, Frigid Units Inc.). Water temperatures were controlled by opposition of cooling and heating units governed by duty-operated controllers of local design and construction. This system maintained water temperatures within ± 1C of the values cited. Initially trout were kept at water temperature equivalent to those in the hatchery; the acclimation process being initiated after a 7-day recovery from transport stresses.

Animals were fed once daily before noon on Purina trout chow. After feeding, excess food and fecal material were removed from the tanks by siphoning. The walls of the tanks were scrubbed once weekly.

(2) Acclimation:

Studies were carried out on trout acclimated to 8 combinations of 2 levels of three factors : temperature (5° and 20°C), oxygen availability (normoxia, 70-80% oxygen saturation; hypoxia, 25-35% oxygen saturation) and photoperiod (16L : 8D and 8L : 16D hours). Of these two combinations approximate what might be termed "winter" and "summer" conditions, i.e., 5°C, normoxia, 8L : 16D and 20°C, hypoxia, 16L : 8D. The others were designed to combine all temperature, photoperiod and oxygen levels,

Figure 10 Diagram of the degassing system used in the present study (Modified from Mount, 1964)


To vacuum source
vacuum regulator
screen water diffuser
float valve
flow meter
water inlet
water level
water pump
cooling coil
fish tank

and to permit assessment of interactions between these variables, i.e.,

(1) 20°C, normoxia, 16L : 8D

(2) 20°C, normoxia, 8L : 16D

(3) 20°C, hypoxia, 16L : 8D (nominal 'summer' group)

(4) 20°C, hypoxia, 8L : 16D

(5) 5°C, normoxia, 16L : 8D

(6) 5°C, normoxia, 8L : 16D (nominal 'winter' group)

(7) 5°C, hypoxia, 16L : 8D

(8) 5°C, hypoxia, 8L : 16D

Animals were brought to their final acclimation temperatures ($5^{o}C$, $20^{o}C$) at a rate of $1^{o}C$ per day. Temperatures were measured daily by M99 Electro : therm digital thermometer. The circulating system provided approximately 75% oxygen saturation without supplementary aeration. A Mount-Brungs vacuum degasser (Mount, 1961, 1964) was used to establish hypoxic conditions (Figure 10). An oilless vacuum pump (1022-103-G272x, Gast MFG. Corp., Michigan) was used to evacuate the degassing cylinder. Water was pumped into this at a rate of 100 $ml.min^{-1}$ and passed over a series of stainless steel grids to increase the degassing surface area. Oxygen content in the water delivered to test tanks was maintained at 25-35% by means of a vacuum gauge. By regulating water inflow and outflow rates outflow water oxygen content ranged from 25-35% saturation. This was pumped into two plastic tanks ($54 \times 26 \times 22\ cm^{3}$) covered with glass plates. The latter were positioned to allow overflow at the downstream end into the main holding trough. Oxygen levels were checked every day in both "normoxic" and "hypoxic" tanks using an oxygen meter (51A, Yellow Springs Instrument).

Each tank was also equipped with a photoperiod hood which employed an automatic time control system (T101-70, Intermatic Incorp., Mississauga, Ontario) to regulate photoperiod. Two 40-watt light bulbs were used in each hood. Water pH was determined daily using a Fisher

Model 140 pH meter. All test groups were acclimated for a minimum of 3 weeks before sampling.

(3) Blood sampling procedure:

To avoid possible artefacts arising from chemical anaesthetization, individual fish was stunned by a blow to the head, and the scales posterior to the anal fin scraped off by scapel. Blood was withdrawn from the caudal vessels into 3cc. plastipak syring treated with 0.1% ammonium heparin (Sigma Chemical Co., St. Louis, Mo., 50,000 units) and provided with an heparinized 23 gauge needle.

Hematocrit and hemoglobin content were measured on freshly-drawn blood, while fresh hemolysates were used for electrophoretic separation. Samples stored in individual, capped vials at $-76^{\circ}C$ were used for ion measurements. Previous studies (Houston and Smeda, 1979) have shown that plasma and packed red cell samples can be stored in this way for several months without significant compositional change.

(4) Hematological determinations:

(a) Hematocrit, Hct (%)

Packed cell volumes were immediately determined in duplicate using Fisher microhematocrit capillary tubes (Drummond Scientific Co., Penna). The blood was drawn into the capillary tubes and centrifuged at 7000 r.p.m. for 5 minutes using an Adams microhematocrit centrifuge. Hematocrit values were determined by means of an Adams microhematocrit-reader.

(b) Blood hemoglobin content, Hb ($g.100\ ml^{-1}$)

The cyanomethemoglobin method was used for hemoglobin determination. Duplicate 10 µl samples of whole blood were mixed with 5.0 ml of solution (pH 7.0-7.4) containing $K_3Fe(CN)_6$, KCN, KH_2PO_4 and Nonidet P-40. This converted oxygenated hemoglobin to a stable cyanomethemoglobin; KH_2PO_4 maintaining pH values within a 7.0-7.4 range to ensure rapid completion of reactions. Nonidet P-40, nonionic detergent was

used to suppress turbidity and facilitate hemolysis. Boehringer Mannheim Diagnostics standard hemoglobin solution was treated in the same way and used at several concentrations, 8.2, 12.1 and 16.3 $g.100^{-1}ml$ to establish a standard curve. Absorbancy values were determined at 540 nm using a Bausch and Lomb Spectronic 100 spectrophotometer.

(c) Mean corpuscular hemoglobin concentration, MCHC ($g.100^{-1}ml$)

MCHC was calculated from the hematocrit and total hemoglobin content using the relationship

$$MCHC = \frac{Hb \times 100}{Hct}$$

(5) Electrophoresis:

Whole blood was separated by centrifugation at 1200 g for 10 minutes in a Fisher model 59 centrifuge. Plasma was removed with a Pasteur pipette, and plasma at the top of the cell column was absorbed with the tip of a cotton swab. The packed red cells were then washed three times in 0.85% NaCl solution, and hemolysed in two volumes of distilled water. Hemolysates were stabilized by bubbling with carbonmonoxide.

Electrophoresis was carried out using cellulose-acetate strips (Titan III-H, Helena Laboratories) in conjunction with Supre-Heme buffer (Tris-EDTA-Boric Acid Buffer, pH 8.2-8.6, 0.025 ionic strength). All electrophoretic separations were carried out at 2°-5°C in a cold room at 400 volts for 20 minutes. After separation, strips were stained with Ponceau-S and washed in a series of 5% acetic acid-methanol baths to remove excess stain. They were then cleared in Clear Aid reagent (polyethalene glycol) and air-dried. Final oven drying was carried out at 70°-75°C to transparentize the strips.

Hemoglobin isomorph mobilities were determined in relation to that of coelectrophoresed human Hb A1 (AA_2 hemocontrol, Helena Laboratories). Densitometric tracings were obtained by scanning at 525 nm with a Helena Laboratories Model 1202 Auto Scanner.

(6) Magnesium determination:

Freshly-drawn blood was separated at 2500 g for 10 minutes in a Fisher centrifuge, plasma being removed by Pasteur pipette. Both plasma and packed cell samples were stored as previously noted before analysis.

In the case of red cell Mg^{+2}, 50 µl of packed cells were hemolyzed in 5 ml of distilled water and stored in the refrigerator (2°C) for 1 hour. 5 ml of 15.2900 $g.lit^{-1}$ $SrCl_2.6H_2O$ (BDH Chemicals Ltd., Poole, England) was then added. Cellular membrane debris was removed by centrifugation in a refrigerated IEC centrifuge at 3400 r.p.m. for 20 minutes. In the case of the plasma, 100 µl of plasma was added into 5 ml of 7.7591 $g.lit^{-1}$ $SrCl_2.6H_2O$, shaken and treated in the same manner.

Standards (BDH) were prepared with identical $SrCl_2$ levels. Analyses were carried out by atomic absorption spectrophotometry using a Perkin-Elmer Model 372 AAS, the instrument being blanked with 7.607 $g.lit^{-1}$ $SrCl_2.6H_2O$ solution between each measurement. To check for drift, a series of 5 standards were measured between duplicated samples. Sample ion concentrations were determined from the standard curve. To check the efficiency of the instrument, versatol (General Diagnostics, New Jersey), an artificial human serum was used.

(7) Chloride determination:

10 µl packed cell samples were pipetted into 2 ml of HNO_3/CH_3COOH reagent solution, and 20 µl samples of plasma were prepared in the same way. Cell samples were held at 2°C in a refrigerator for 12-24 hours before analysis to facilitate completion of hemolysis. Plasma, blanks, versatol and standards were treated in the same manner.

A Buchler-Cotlove chloridometer was used for chloride determinations. Prior to analysis, 2 drops of gelatin reagent (0.62 g of a reagent containing 60:1:1 gelatin: thymol blue: thymol/100 ml distilled

water) were added to each sample vial. The chloridometer system utilizes passage of a constant direct current between a pair of silver electrodes. This prompts release of silver ions at constant rate into solution. The end-point is reached after all Cl^- has been precipitated in the form of $AgCl_2$. Chloride concentrations were calculated from the following relationship.

$$\text{sample concentration } (mmol.lit^{-1}) = \frac{Ts - Tb}{Tstd - Tb} \times \text{Std. concentration}$$

where; Ts = titration time for unknown sample (sec)

Tb = titration time for blank (sec)

Tstd = titration time for standard (sec)

Std. concentration = 3.9989 $mmol.lit^{-1}$

(8) Trapped plasma factor:

Mg^{+2} and Cl^- determinations in packed red cells involve an error due to plasma entrapment within the interstices of the packed cell column. Accordingly, intraerythrocytic ion levels were corrected for trapped plasma using the equation (Houston and Smeda, 1979).

$$\text{Corrected ion level } (mmol.lit^{-1}) = \frac{(E) - (P \times 0.0282)}{0.9718}$$

where; E = uncorrected ion level $(mmol.lit^{-1})$

P = ion level in plasma $(mmol.lit^{-1})$

(9) Statistical analysis:

Means, standard errors of the mean, standard deviations, variances and 95% confidence intervals were calculated for all samples using procedures described by Sokal and Rholf (1969) on an Apple II computer. Significance tests were carried out by three-way factorial anova (ANOVA 3). Prior to ANOVA, data for hemoglobin contents, ion concentrations and hemoglobin fraction concentrations were transformed to base-10 logarithms. Arcsine transformation was carried out for hematocrit, ion:Hb and percentage concentrations of hemoglobin components.

Significance was attributed to differences at the 0.05 level or better.

To identify the number of statistically distinct polymorphs graphical analysis of the 95% confidence limits of mean Rx values was used. Graphical analysis was carried out by using the following criteria.

(1) If the 95% confidence interval for one sample overlaps the mean of the second sample, the samples almost certainly do not differ at the 0.05 level.

(2) If the confidence intervals are of roughly the same magnitude and do not overlap, the samples almost certainly do differ at the 0.05 level.

(3) If the confidence intervals are not of the same magnitude and we can replace the smaller with the larger without overlap, the samples almost certainly do differ at the 0.05 level.

RESULTS

Acclimatory Variations In Hematological Indices:

Observations on the hematological indices considered in this study (i.e., hemoglobin content, hematocrit, mean corpuscular hemoglobin concentration) are summarized, with the outcome of analyses of variance in Table 2. All values for each specimen are summarized in Appendix Tables 1 to 8.

Temperature: Significant, but not pronounced thermal effects were evident; animals at 20°C being characterized by higher hemoglobin levels than were those acclimated to 5°C. This was, not unexpectedly, also the case with hematocrit as well. Mean erythrocytic hemoglobin concentrations were not, however, influenced by temperature. This suggests that increases in hemoglobin and hematocrit reflect increased numbers of red cells (i.e., erythropoietic stimulation) rather than increased hemoglobin synthesis by cells already in the circulation.

Oxygen: Oxygen availability also influenced hemoglobin and hematocrit; exposure to hypoxic circumstances being associated with elevation of both indices. Comparison of mean values, and significance levels arising from variance analysis indicated that, under circumstances imposed, exposure to reduced oxygen had a more profound effect on blood O_2-carrying capacity than did increased temperature. Again, there was little change in MCHC, and this also pointed to the greater likelihood of erythropoietic activity than of hemoglobin synthesis in circulating cells.

Photoperiod: Interestingly, reduced day length was also associated with increases in hemoglobin and hematocrit, but again, there was little alteration in MCHC.

Although each factor considered had a significant effect on hemoglobin and hematocrit, significant interactions between them were,

Table 2 Summary of hematological measurements.

Reported as the mean ± standard error of the mean

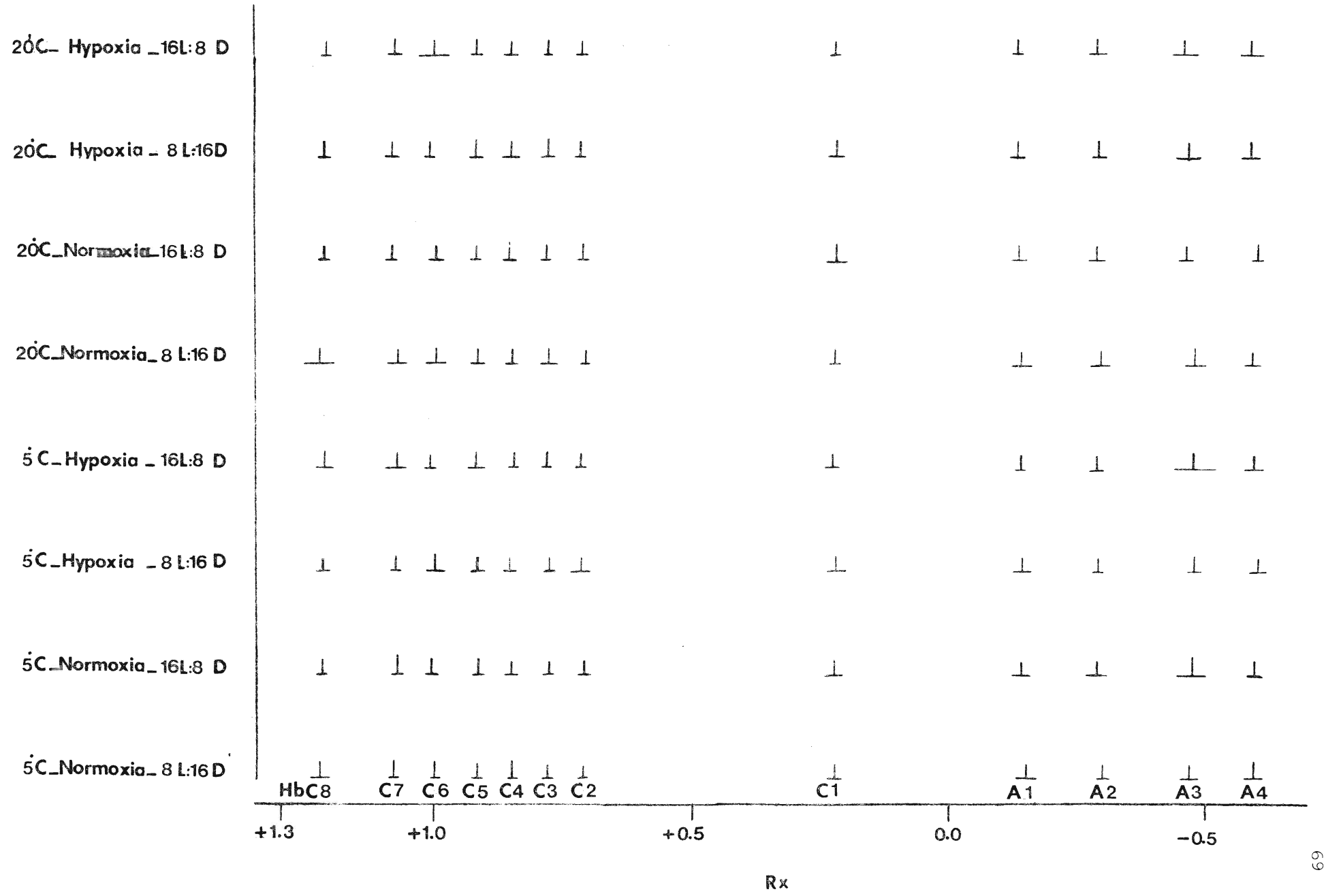
(95% confidence intervals)

Experimental Conditions			Measurements		
Temp ^{o}C	Oxygen %	Light hr	Hb $g.dl^{-1}$	Hct %	MCHC $g.dl^{-1}$
20	25-35 (Hypoxia)	16L: 8D	7.4 ± 0.3 (6.7 - 8.1)	36.5 ± 1.3 (33.4 - 39.5)	20.28 ± 0.31 (19.60 - 20.97)
20	25-35 (Hypoxia)	8L:16D	9.8 ± 0.4 (8.7 -10.8)	45.9 ± 1.5 (42.4 - 49.3)	21.40 ± 0.65 (19.96 - 22.83)
20	70-80 (Normoxia)	16L: 8D	6.7 ± 0.4 (5.7 - 7.8)	32.4 ± 2.2 (27.4 - 37.4)	20.90 ± 0.42 (19.97 - 21.83)
20	70-80 (Normoxia)	8L:16D	7.7 ± 0.3 (6.9 - 8.5)	34.1 ± 2.5 (28.4 - 39.7)	23.43 ± 1.28 (20.60 - 26.25)
5	25-35 (Hypoxia)	16L: 8D	7.2 ± 0.5 (6.0 - 8.4)	32.4 ± 2.5 (26.8 - 38.1)	22.94 ± 1.66 (19.27 - 26.60)
5	25-35 (Hypoxia)	8L:16D	8.7 ± 0.3 (7.9 - 9.4)	41.2 ± 2.0 (36.7 - 45.6)	21.33 ± 0.42 (20.39 - 22.26)
5	70-80 (Normoxia)	16L: 8D	6.9 ± 0.3 (6.2 - 7.6)	33.0 ± 1.6 (29.3 - 36.7)	21.14 ± 0.49 (20.06 - 22.23)
5	70-80 (Normoxia)	8L:16D	6.3 ± 0.4 (5.2 - 7.3)	28.1 ± 2.2 (23.0 - 33.1)	22.66 ± 0.68 (21.16 - 24.15)
Anova Table					
Temperature			P< 0.05	P< 0.05	N.S.
Oxygen			P< 0.01	P< 0.01	N.S.
Photoperiod			P< 0.01	P< 0.05	N.S.
Temperature x Oxygen			N.S.	N.S.	N.S.
Temperature x Photoperiod			P< 0.05	N.S.	N.S.
Oxygen x Photoperiod			P< 0.01	P< 0.01	N.S.
Temperature x Oxygen x Photoperiod			N.S.	N.S.	N.S.

Figure 11 Relative nobilities (Rx) of rainbow trout hemoglobin isomorphs

Horizontal line; 95% confidence intervals

Vertical line; mean value


20C_ Hypoxia _16L:8 D
20C_ Hypoxia _ 8 L:16D
20C_Normoxia_16L:8 D
20C_Normoxia_ 8 L:16 D
5C_Hypoxia _ 16L:8 D
5C_Hypoxia _ 8 L:16 D
5C_Normoxia_ 16L:8 D
5C_Normoxia_ 8 L:16 D
HbC8
C7
C6
C5
C4
C3
C2
C1
A1
A2
A3
A4
+1.3
+1.0
+0.5
0.0
-0.5
Rx

with one exception, not detected. Oxygen interacted with photoperiod on both hemoglobin and hematocrit.

Of particular interest was the comparison of groups under nominal "summer" and "winter" conditions. The former (20°C-hypoxia-16L:8D) were characterized by significantly higher hemoglobin and hematocrit values than were animals exposed to the 5°C-normoxia-8L:16D regime. These data suggested that, in terms of blood oxygen carrying capacity, the apparently stimulatory effects of heightened temperature and oxygen demand with reduced oxygen availability overrode the influence of extended day length.

Hemoglobin System Organization.

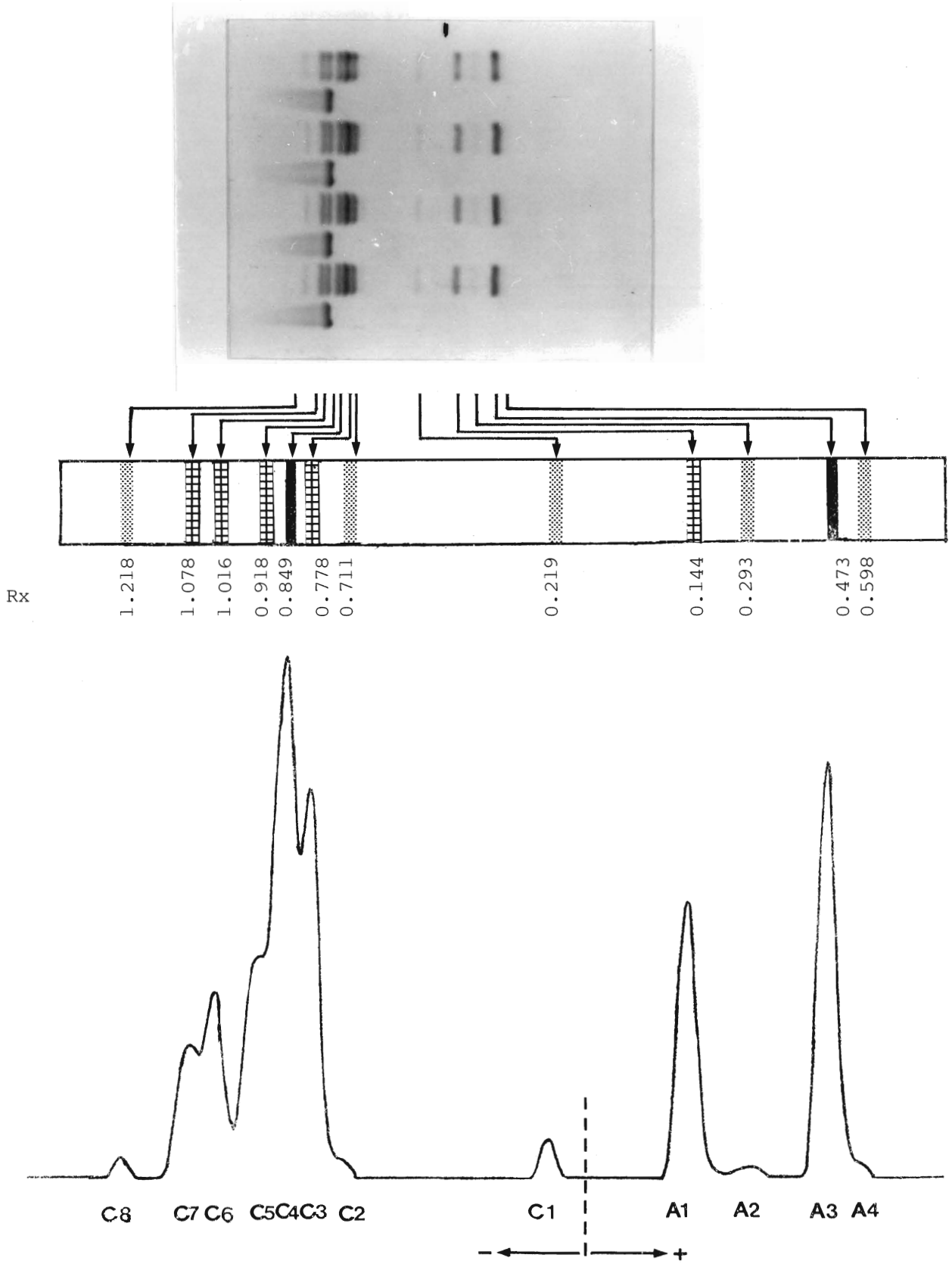
Figure 11 summarizes the distribution of rainbow trout hemoglobin mobilities encountered in the present study relative to that of human HbA1. Twelve isomorphs, designated C(cathode)8 through A(anode)4 were typically observed under the electrophoretic conditions employed. None were eliminated or added under the experimenal conditions imposed. Differences in relative abundancy were apparent (Table 4), and it is convenient to group these isomorphs in order of decreasing representation as major (isomorphs C4, A3), intermediate (isomorphs C3, A1, C5, C6, C7) and minor components (isomorphs A2, C1, C8, C2, A4). Particular emphasis will be given to the former groups in subsequent data treatments since it may be reasonably argued that variations in minor components probably have little real impact upon gas transport capability.

Table 3 and 4 summarize variations in relative (i.e., % of total hemoglobin) and absolute (actual) component abundancies. Again, each table includes the outcome of variance analysis, while individual data items have been compiled in Appendix Tables 18 through 33.

Figure 12 (a) Sample cellulose acetate strip.

(b) Diagram of the above sample strip.

(c) Densitomatric tracing of the strip.


Rx
1.218
1.078
1.016
0.918
0.849
0.778
0.711
0.219
0.144
0.293
0.473
0.598
C8
C7
C6
C5
C4
C3
C2
C1
A1
A2
A3
A4
+

Major Components

The most abundant isomorphs, C4, constituted from 25.2 ± 0.47 to 27.9 ± 0.51% of the total hemoglobin present, and this proportion was not significantly influenced by differences in temperature, oxygen availability or photoperiod per se. One significant interaction was evident: that between temperature and photoperiod.

The next most abundant fraction (A3, 17.2 ± 0.79 - 20.5 ± 0.43% was significantly affected by temperature, decreasing at the higher temperature, but by no other factors or combination of factors.

On the other hand, the actual amounts of these hemoglobins were strongly affected by the variables considered. The amount of isomorphs C4, for example, was significantly elevated at 20°C, under hypoxic condition and with reduced photoperiod. A significant oxygen-photoperiod interaction was also detected. In the case of isomorph A3 temperature had no effect on actual concentration. Significant increases were, however, associated with hypoxia and abbreviated photoperiod. Not unexpectedly a significant interaction between photoperiod and oxygen was evident. This was also the case with temperature and photoperiod interaction.

The elevation in abundancy of C4 in "summer" as compared to "winter" animals is therefore consistent with the effects of temperature and hypoxia which, together, appear to outweigh the influence of reduced photoperiod. A3 concentration was also elevated in "summer" trout. Temperature per se was apparently not a factor in this instance, reduction in oxygen availability being more important in this case, as with isomorph C4, than reduced day length.

Table 3 Summary of percentage contents of hemoglobin components.

Reported as the mean ± standard error of the mean (95% confidence intervals)

Experimental Conditions			Percentage concentrations of Hb components					
Temp. °C	Oxygen %	Light hr.	C4	A3	C3	A1	C5	C6
20	25-35 (Hypoxia)	16L: 8D	27.44 ± 0.50 (26.34 - 28.55)	17.82 ± 0.72 (16.22 - 19.42)	14.67 ± 0.59 (13.36 - 15.98)	10.78 ± 0.44 (9.80 - 11.75)	10.23 ± 0.51 (9.09 - 11.37)	8.55 ± 0.29 (7.91 - 9.18)
20	25-35 (Hypoxia)	8L:16D	26.86 ± 0.34 (26.10 - 27.62)	17.75 ± 0.46 (16.74 - 18.78)	13.34 ± 0.44 (12.36 - 14.32)	10.57 ± 0.47 (9.52 - 11.61)	10.37 ± 0.38 (9.52 - 11.23)	8.99 ± 0.21 (8.53 - 9.46)
20	70-80 (Normoxia)	16L: 8D	27.41 ± 0.77 (25.71 - 29.11)	17.20 ± 0.79 (15.45 - 18.95)	15.68 ± 1.10 (13.25 - 18.10)	9.67 ± 0.46 (8.65 - 10.7)	9.95 ± 0.37 (9.13 - 10.77)	8.14 ± 0.39 (7.27 - 9.01)
20	70-80 (Normoxia)	8L:16D	27.10 ± 0.42 (26.18 - 28.03)	18.72 ± 1.01 (16.43 - 20.96)	15.20 ± 0.74 (13.57 - 16.83)	10.65 ± 0.57 (9.39 - 11.91)	10.24 ± 0.33 (9.50 - 10.98	9.12 ± 0.26 (8.54 - 9.70)
5	25-35 (Hypoxia)	16L: 8D	25.23 ± 0.47 (24.19 - 26.26)	19.60 ± 0.34 (18.84 - 20.36)	15.27 ± 0.64 (13.84 - 16.69)	7.94 ± 0.72 (6.34 - 9.54)	11.28 ± 0.62 (9.89 - 12.66)	8.73 ± 0.25 (8.16 - 9.29)
5	25-35 (Hypoxia)	8L:16D	27.20 ± 0.55 (25.98 - 28.42)	19.95 ± 0.69 (18.41 - 21.49)	14.28 ± 0.41 (13.36 - 15.20)	7.24 ± 0.89 (5.28 - 9.20)	10.14 ± 0.44 (9.16 - 11.11)	8.60 ± 0.27 (8.0 - 9.20)
5	70-80 (Normoxia)	16L: 8D	26.25 ± 0.47 (25.21 - 27.28)	20.47 ± 0.43 (19.51 - 21.44)	14.12 ± 0.67 (12.63 - 15.62)	11.25 ± 0.59 (9.94 - 12.56)	10.35 ± 0.54 (9.15 - 11.56)	10.35 ± 0.54 (9.15 - 11.56)
5	70-80 (Normoxia)	8L:16D	27.90 ± 0.51 (26.78 - 29.03)	19.44 ± 0.47 (18.41 - 20.48)	13.90 ± 0.48 (12.83 - 14.97)	11.38 ± 0.54 (10.18 - 12.58)	8.84 ± 0.54 (7.65 - 10.05)	8.84 ± 0.54 (7.63 - 10.05)
Anova Table								
Temperature			N.S.	P<0.01	N.S.	P<0.05	N.S.	N.S.
Oxygen			N.S.	N.S.	N.S.	P<0.01	N.S.	N.S.
Photoperiod			N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Temperature x Oxygen			N.S.	N.S.	P<0.05	P<0.01	N.S.	N.S.
Temperature x Photoperiod			P<0.01	N.S.	N.S.	N.S.	P<0.05	P<0.05
Oxygen x Photoperiod			N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Temperature x Oxygen x Photoperiod			N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Table 3 (Cont'd..)

Experimental Conditions			Percentage concentrations of Hb components					
Temp. °C	Oxygen %	Light hr.	C7	A2	C1	C8	C2	A4
20	25-35 (Hypoxia)	16L: 8D	6.59 ± 0.38 (5.74 - 7.44)	1.06 ± 0.24 (0.52 - 1.60)	1.07 ± 0.17 (0.68 - 1.46)	0.65 ± 0.08 (0.47 - 0.82)	0.54 ± 0.11 (0.28 - 0.80)	0.34 ± 0.07 (0.18 - 0.50)
20	25-35 (Hypoxia)	8L:16D	7.36 ± 0.20 (6.92 - 7.80)	1.72 ± 0.28 (1.09 - 2.34)	0.98 ± 0.12 (0.71 - 1.26)	0.84 ± 0.08 (0.66 - 1.02)	0.87 ± 0.09 (0.65 - 1.09)	0.69 ± 0.06 (0.54 - 0.85)
20	70-80 (Normoxia)	16L: 8D	6.05 ± 0.53 (4.84 - 7.24)	1.63 ± 0.21 (1.15 - 2.11)	0.83 ± 0.12 (0.55 - 1.10)	1.02 ± 0.10 (0.80 - 1.25)	0.99 ± 0.15 (0.66 - 1.33)	0.85 ± 0.11 (0.59 - 1.11)
20	70-80 (Normoxia)	8L:16D	6.79 ± 0.25 (6.22 - 7.36)	1.28 ± 0.24 (0.75 - 1.82)	0.78 ± 0.11 (0.53 - 1.03)	0.85 ± 0.06 (0.69 - 1.00)	0.75 ± 0.19 (0.32 - 1.18)	0.53 ± 0.09 (0.32 - 0.74)
5	25-35 (Hypoxia)	16L: 8D	7.22 ± 0.39 (6.34 - 8.09)	1.24 ± 0.12 (0.96 - 1.51)	0.97 ± 0.11 (0.71 - 1.23)	0.97 ± 0.11 (0.73 - 1.22)	1.04 ± 0.10 (0.80 - 1.27)	1.15 ± 0.11 (0.89 - 1.41)
5	25-35 (Hypoxia)	8L:16D	7.18 ± 0.37 (6.36 - 8.0)	1.07 ± 0.10 (0.84 - 1.30)	1.04 ± 0.09 (0.83 - 1.24)	0.93 ± 0.07 (0.76 - 1.10)	0.85 ± 0.13 (0.56 - 1.14)	0.71 ± 0.12 (0.43 - 0.99)
5	70-80 (Normoxia)	16L: 8D	7.14 ± 0.44 (6.16 - 8.11)	1.27 ± 0.23 (0.74 - 1.80)	0.73 ± 0.10 (0.51 - 0.95)	0.90 ± 0.08 (0.71 - 1.10)	0.57 ± 0.05 (0.44 - 0.70)	0.56 ± 0.05 (0.44 - 0.67)
5	70-80 (Normoxia)	8L:16D	5.72 ± 0.32 (5.01 - 6.44)	1.79 ± 0.23 (1.27 - 2.31)	1.01 ± 0.18 (0.60 - 1.42)	0.63 ± 0.09 (0.42 - 0.85)	0.72 ± 0.07 (0.55 - 0.89)	0.76 ± 0.13 (0.47 - 1.05)
Anova Table								
Temperature			N.S.	N.S.	N.S.	N.S.	N.S.	P<0.01
Oxygen			P<0.05	N.S.	N.S.	N.S.	N.S.	N.S.
Photoperiod			N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Temperature x Oxygen			N.S.	N.S.	N.S.	P<0.01	P<0.05	P<0.01
Temperature x Photoperiod			P<0.05	N.S.	N.S.	N.S.	N.S.	N.S.
Oxygen x Photoperiod			N.S.	N.S.	N.S.	P<0.05	N.S.	N.S.
Temperature x Oxygen x Photoperiod			N.S.	P<0.01	N.S.	N.S.	P<0.01	P<0.01

Table 4 Summary of hemoglobin polymorph contents ($g.dl^{-1}$).

Reported as the mean ± standard error of the mean (95% confidence intervals)

Experimental Conditions			Actual amounts of Hb components $g.dl^{-1}$					
Temp. °C	Oxygen %	Light hr.	C4	A3	C3	A1	C5	C6
20	25-35 (Hypoxia)	16L: 8D	2.02 ± 0.07 (1.86 - 2.19)	1.33 ± 0.08 (1.13 - 1.52)	1.07 ± 0.04 (0.97 - 1.18)	0.80 ± 0.05 (0.68 - 0.93)	0.75 ± 0.03 (0.66 - 0.83)	0.63 ± 0.03 (0.55 - 0.72)
20	25-35 (Hypoxia)	8L:16D	2.64 ± 0.15 (2.31 - 2.97)	1.74 ± 0.09 (1.53 - 1.95)	1.29 ± 0.02 (1.22 - 1.35)	1.03 ± 0.06 (0.89 - 1.17)	1.01 ± 0.05 (0.88 - 1.14)	0.88 ± 0.03 (0.79 - 0.96)
20	70-80 (Normoxia)	16L: 8D	1.87 ± 0.16 (1.50 - 2.24)	1.16 ± 0.08 (0.96 - 1.35)	1.08 ± 0.13 (0.79 - 1.36)	0.65 ± 0.04 (0.54 - 0.76)	0.67 ± 0.04 (0.56 - 0.77)	0.54 ± 0.03 (0.46 - 0.62)
20	70-80 (Normoxia)	8L:16D	2.10 ± 0.11 (1.84 - 2.35)	1.45 ± 0.11 (1.19 - 1.70)	1.18 ± 0.08 (0.99 - 1.36)	0.77 ± 0.04 (0.66 - 0.87)	0.79 ± 0.04 (0.68 - 0.90)	0.70 ± 0.03 (0.62 - 0.78)
5	25-35 (Hypoxia)	16L: 8D	1.82 ± 0.14 (1.50 - 2.15)	1.42 ± 0.12 (1.15 - 1.69)	1.09 ± 0.10 (0.87 - 1.32)	0.58 ± 0.07 (0.41 - 0.76)	0.80 ± 0.06 (0.66 - 0.94)	0.63 ± 0.05 (0.51 - 0.75)
5	25-35 (Hypoxia)	8L:16D	2.37 ± 0.10 (2.14 - 2.60)	1.74 ± 0.09 (1.53 - 1.96)	1.24 ± 0.06 (1.09 - 1.40)	0.64 ± 0.09 (0.42 - 0.86)	0.88 ± 0.04 (0.77 - 0.98)	0.75 ± 0.04 (0.66 - 0.84)
5	70-80 (Normoxia)	16L: 8D	1.82 ± 0.08 (1.64 - 2.0)	1.41 ± 0.05 (1.28 - 1.54)	0.99 ± 0.07 (0.82 - 1.15)	0.78 ± 0.06 (0.65 - 0.92)	0.71 ± 0.03 (0.62 - 0.79)	0.63 ± 0.03 (0.54 - 0.72)
5	70-80 (Normoxia)	8L:16D	1.75 ± 0.12 (1.47 - 2.02)	1.22 ± 0.09 (1.0 - 1.44)	0.87 ± 0.07 (0.71 - 1.02)	0.73 ± 0.07 (0.56 - 0.90)	0.55 ± 0.05 (0.43 - 0.67)	0.52 ± 0.04 (0.42 - 0.63)
Anova Table								
Temperature			P<0.05	N.S.	P<0.05	P<0.01	P<0.05	N.S.
Oxygen			P<0.01	P<0.01	P<0.01	N.S.	P<0.01	P<0.01
Photoperiod			P<0.01	P<0.01	N.S.	N.S.	N.S.	P<0.01
Temperature x Oxygen			N.S.	N.S.	N.S.	P<0.01	N.S.	N.S.
Temperature x Photoperiod			N.S.	P<0.05	N.S.	N.S.	P<0.01	P<0.01
Oxygen x Photoperiod			P<0.01	P<0.05	N.S.	N.S.	P<0.01	P<0.05
Temperature x Oxygen x Photoperiod			N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Table 4 (Cont'd..)

Experimental conditions			Actual amounts of Hb components $g.dl^{-1}$					
Temp. °C	Oxygen %	Light hr.	C7	A2	C1	C8	C2	A4
20	25-35 (Hypoxia)	16L: 8D	0.49 ± 0.04 (0.40 - 0.58)	0.08 ± 0.02 (0.03 - 0.12)	0.08 ± 0.01 (0.05 - 0.11)	0.05 ± 0.01 (0.03 - 0.06)	0.04 ± 0.01 (0.02 - 0.05)	0.02 ± 0.01 (0.01 - 0.03)
20	25-35 (Hypoxia)	8L:16D	0.72 ± 0.04 (0.63 - 0.81)	0.17 ± 0.02 (0.10 - 0.23)	0.09 ± 0.01 (0.06 - 0.13)	0.08 ± 0.01 (0.05 - 0.11)	0.08 ± 0.01 (0.06 - 1.04)	0.07 ± 0.01 (0.05 - 0.08)
20	70-80 (Normoxia)	16L: 8D	0.39 ± 0.03 (0.32 - 0.46)	0.10 ± 0.01 (0.07 - 0.13)	0.05 ± 0.01 (0.03 - 0.07)	0.06 ± 0.01 (0.05 - 0.08)	0.06 ± 0.01 0.04 - 0.09)	0.05 ± 0.01 0.03 - 0.07)
20	70-80 (Normoxia)	8L:16D	0.52 ± 0.03 (0.45 - 0.59)	0.08 ± 0.01 (0.05 - 0.12)	0.06 ± 0.01 (0.04 - 0.07)	0.06 ± 0.01 (0.05 - 0.07)	0.06 ± 0.01 (0.02 - 0.09)	0.03 ± 0.01 (0.02 - 0.04)
5	25-35 (Hypoxia)	16L: 8D	0.52 ± 0.04 (0.41 - 0.62)	0.09 ± 0.01 (0.06 - 0.11)	0.07 ± 0.01 (0.04 - 0.09)	0.07 ± 0.01 (0.05 - 0.09)	0.07 ± 0.01 (0.05 - 0.09)	0.08 ± 0.01 (0.05 - 0.11)
5	25-35 (Hypoxia)	8L:16D	0.62 ± 0.03 (0.55 - 0.69)	0.09 ± 0.01 (0.07 - 0.11)	0.09 ± 0.01 (0.07 - 0.11)	0.08 ± 0.01 (0.06 - 0.09)	0.07 ± 0.01 (0.05 - 0.10)	0.06 ± 0.01 (0.03 - 0.08)
5	70-80 (Normoxia)	16L: 8D	0.49 ± 0.03 (0.41 - 0.57)	0.09 ± 0.01 (0.05 - 0.13)	0.05 ± 0.01 (0.03 - 0.07)	0.06 ± 0.01 (0.05 - 0.07)	0.04 ± 0.01 (0.03 - 0.05)	0.04 ± 0.01 (0.03 - 0.05)
5	70-80 (Normoxia)	8L:16D	0.36 ± 0.03 (0.29 - 0.42)	0.10 ± 0.01 (0.07 - 0.14)	0.06 ± 0.01 (0.03 - 0.09)	0.04 ± 0.01 (0.02 - 0.05)	0.04 ± 0.01 (0.03 - 0.05)	0.04 ± 0.01 (0.03 - 0.06)
Anova Table								
Temperature			N.S.	N.S.	N.S.	N.S.	N.S.	P<0.05
Oxygen			P<0.01	N.S.	P<0.01	N.S.	P<0.05	P<0.05
Photoperiod			P<0.01	N.S.	N.S.	N.S.	N.S.	N.S.
Temperature x Oxygen			N.S.	N.S.	N.S.	P<0.01	N.S.	P<0.01
Temperature x Photoperiod			P<0.01	N.S.	N.S.	P<0.05	N.S.	P<0.05
Oxygen x Photoperiod			P<0.01	N.S.	N.S.	P<0.01	P<0.05	P<0.05
Temperature x Oxygen x Photoperiod			P<0.05	P<0.01	N.S.	N.S.	P<0.01	P<0.01

Components of Itermediate Abundance

The principal component of those grouped in the intermediate category was isomorph C3 which constituted 13.3 ± 0.45 to 15.7 ± 1.1% of total hemoglobin. As was the case with C4, temperature, oxygen content and photoperiod individually had little effect on relative abundancy. The actual concentration of this fraction was, however, elevated at higher temperatures and under hypoxic conditions; effects consistent with the observed concentration difference between "summer" (1.07 ± 0.04 $g.dl^{-1}$) and "winter" trout (0.87 ± 0.07 $g.dl^{-1}$).

Isomorph C5 made up 8.8 ± 0.54 to 11.3 ± 0.62% of total hemoglobin: a proportion not significantly influenced by the variables considered. Like C3, however, the absolute abundance of this fraction increased significantly with reduction in oxygen availability. Thermal effects were not pronounced. Variations in photoperiod had no detectable influence, and the difference between "summer" and "winter" animals was consistent with response to oxygen availability. Interactions between photoperiod and temperature and oxygen content were significant.

The third most abundant of these fractions was isomorph C6 (8.1 ± 0.39 - 10.4 ± 0.55%). No factor had significant impact upon its relative abundance. Temperature was also without influence on absolute concentration. Highly significant increases in absolute C6 content were, however, associated with exposure to hypoxia and reduction in day length. This suggests that the relatively higher levels of this isomorph in "summer" (0.63 ± 0.03 $g.dl^{-1}$) than in "winter" fish (0.52 ± 0.04 $g.dl^{-1}$) were attributable to hypoxic influence since photoperiodic effects would reverse this.

Isomorph A1 (7.2 ± 0.89 - 11.4 ± 0.54%), unlike most of these considered did show proportional changes in relation to the conditions imposed in that significant ($P<0.01$) reductions were encountered with exposure to hypoxia. Interestingly, absolute concentrations were influenced only by

temperature. Here, however, the effect of temperature was both substantial and consistent with the difference between "summer" and "winter" trout. Only one significant interaction, that between temperature and oxygen content was evident and this influenced both relative and absolute Al levels.

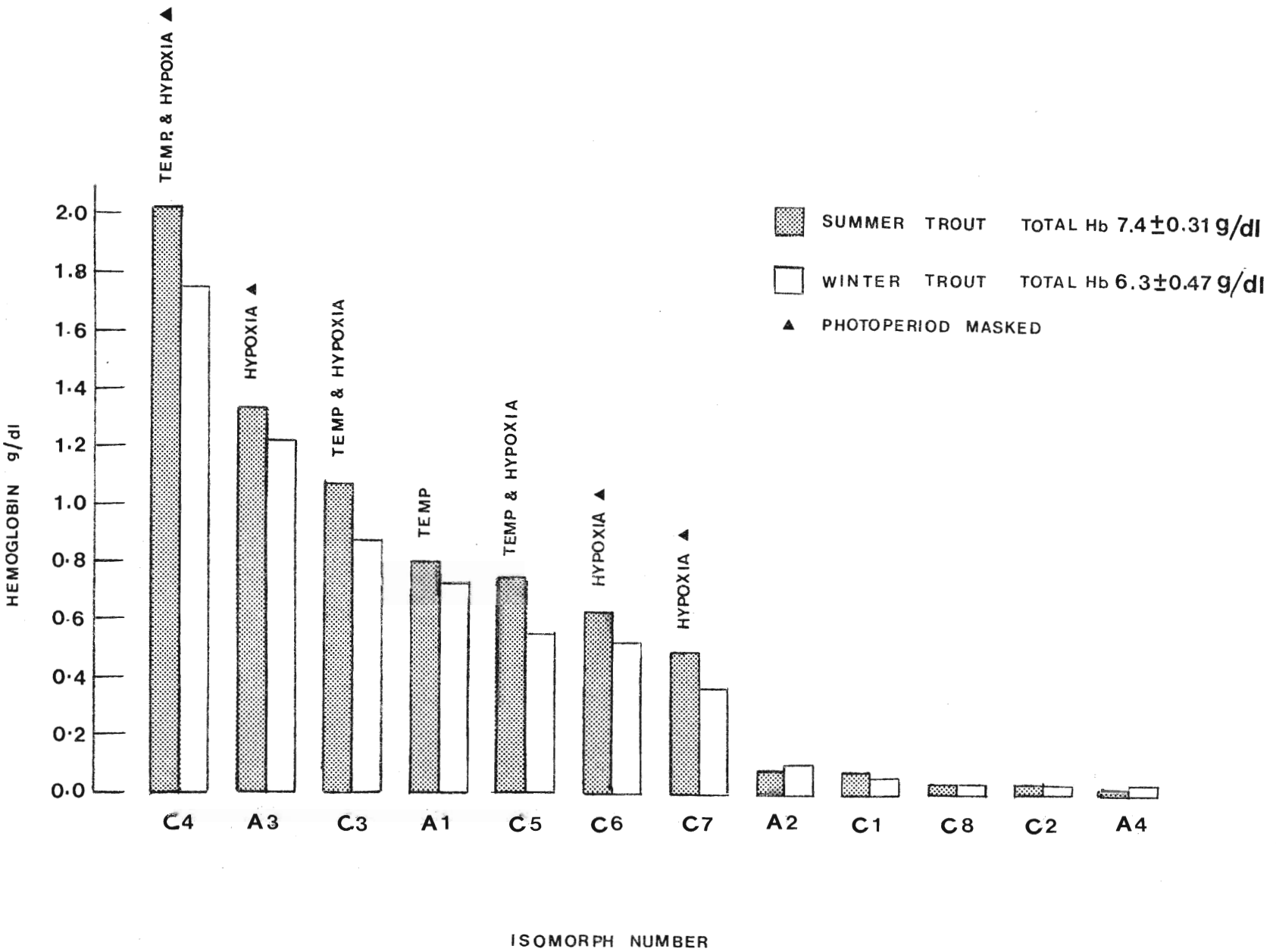
The final component of this group, C7, represented from 5.7 ± 0.32 to 7.4 ± 0.20% of total hemoglobin. A barely significant increase in the proportional abundance of this fraction was associated with reduction in oxygen availability. This was true of the actual concentration of C7 as well. Significant increases in concentration were also associated with reduced day length. Significant interactions between photoperiod, and temperature and oxygen availability were apparent in relation to actual concentration. The higher concentration (0.49 ± 0.04 $g.dl^{-1}$) characteristic of "summer" as compared to "winter" fish presumably reflected the stronger influence of hypoxia.

Minor components

The major fractions, C4 and A3, constituted from 42.4 to 48.4% of total hemoglobin. Fractions of intermediate abundancy (C3, Al, C5, C6, C7) collectively accounted for 43.1 to 55.7%. The remaining isomorphs (A2, Cl, C8, C2, A4) together represented only 3.2 to 6.1% of the total. Only one significant environmental effect on relative abundance was apparent; that of temperature on A4. Half of these fractions (C2, Cl, A4) also exhibited some modest response in actual abundancies to oxygen tension, none to photoperiod and, as would be expected, A4 was unique in exhibiting thermal influence.

In general, their proportional abundancies were less influenced by the factors considered than were actual isomorph concentrations. In terms of proportional abundancy, 29% of the major and intermediate isomorphs

Figure 13 Actual concentrations of isomorphs in "summer" and "winter" trout.


TEMP. & HYPOXIA ◀
HYPOXIA ◀
TEMP & HYPOXIA
TEMP
TEMP & HYPOXIA
HYPOXIA ◀
HYPOXIA ◀
HEMOGLOBIN g/dl
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0·0
SUMMER TROUT TOTAL Hb 7.4±0.31 g/dl
WINTER TROUT TOTAL Hb 6.3±0.47 g/dl
▲ PHOTOPERIOD MASKED
C4
A3
C3
A1
C5
C6
C7
A2
C1
C8
C2
A4
ISOMORPH NUMBER

were significantly influenced by temperature *per se* and/or oxygen content *per se*; none by photoperiod. By reference to absolute concentration significant oxygen effect was apparent in 85% of the major and intermediate fractions, and like the effect of photoperiod, significant thermal influence was apparent in 57% of comparisons.

Significant interactions between oxygen availability and photoperiod was not observed in relation to the relative abundancies of these isomorphs. Temperature x photoperiod interaction seemed to be the most prevalent since one major and 3 intermediate fractions were influenced in this way. Temperature x oxygen interactions did not affect either major components, but did influence the intermediate isomorphs C3 and A1. Three-factor interactions were not prevalent and did not involve any major and intermediate isomorphs. Actual abundancies were also independent of 3 factor interaction. The interactions between photoperiod and temperature, and oxygen availability were quite similar and significant in relation to isomorphs A3, C5, C6 and C7. It should be emphasized, however, that comparisons of animals under nominal "summer" and "winter" circumstances frequently suggested that potential photoperiodic influences were normally masked by the effects of temperature or oxygen availability or both (Figure 13).

Plasma and Red Cell Tonic Composition

The outcome of plasma and erythrocytic Cl^- and Mg^{+2} determinations is summarized, with the results of analysis of variance in Table 5. Individual values for these parameters are summarized in Appendix Tables 1 through 8.

Temperature

Temperature had a highly significant influence upon all four variables. Increases in both plasma and red cell Cl^- concentrations were associated with exposure to the higher temperature. This was true as well of plasma magnesium. By contrast, red cell magnesium levels were

5. Summary of ion levels. Reported as the mean ± standard error of the mean (95% confidence intervals)

Experimental Conditions			Measurements			
Temp. °C	Oxygen %	Light hr.	pCl^{-} $mmol.l^{-1}$	$rbcCl^{-}$ $mmol.l^{-1}$	pMg^{+2} $mmol.l^{-1}$	$rbcMg^{+2}$ $mmol.l^{-1}$
20	25-35 (Hypoxia)	16L: 8D	124.4 ± 1.3 (121.5 - 127.3)	75.2 ± 1.4 (71.9 - 78.5)	1.06 ± 0.04 (0.96 - 1.15)	4.15 ± 0.16 (3.78 - 4.51)
20	25-35 (Hypoxia)	8L:16D	133.7 ± 2.1 (128.9 - 138.4)	72.5 ± 1.5 (69.2 - 75.8)	0.93 ± 0.02 (0.88 - 0.99)	4.40 ± 0.11 (4.14 - 4.65)
20	70-80 (Normoxia)	16L: 8D	124.7 ± 3.0 (118.0 - 131.3)	83.5 ± 2.0 (79.0 - 88.0)	0.94 ± 0.03 (0.86 - 1.01)	4.18 ± 0.18 (3.78 - 4.58)
20	70-80 (Normoxia)	8L:16D	128.3 ± 3.3 (120.8 - 135.7)	75.2 ± 2.4 (69.8 - 80.6)	0.93 ± 0.02 (0.87 - 0.99)	4.75 ± 0.28 (4.13 - 5.38)
5	25-35 (Hypoxia)	16L: 8D	111.7 ± 4.1 (102.5 - 120.9)	58.7 ± 1.5 (55.3 - 62.2)	0.86 ± 0.09 (0.65 - 1.06)	5.75 ± 0.10 (5.52 - 5.99)
5	25-35 (Hypoxia)	8L:16D	124.1 ± 3.1 (117.2 - 131.0)	65.0 ± 2.2 (59.9 - 70.0)	0.79 ± 0.02 (0.72 - 0.85)	4.90 ± 0.09 (4.68 - 5.12)
5	70-80 (Normoxia)	16L: 8D	126.4 ± 1.9 (122.0 - 130.8)	54.7 ± 1.3 (51.8 - 57.7)	0.81 ± 0.02 (0.74 - 0.83)	6.69 ± 0.14 (6.38 - 7.0)
5	70-80 (Normoxia)	8L:16D	121.9 ± 1.9 (117.7 - 126.1)	59.8 ± 1.8 (55.8 - 63.9)	0.72 ± 0.03 (0.65 - 0.78)	6.14 ± 0.17 (5.74 - 6.53)
Anova Table						
Temperature			P<0.01	P<0.01	P<0.01	P<0.01
Oxygen			N.S.	N.S.	P<0.05	P<0.01
Photoperiod			P<0.05	N.S.	P<0.05	N.S.
Temperature x Oxygen			P<0.05	P<0.01	N.S.	P<0.01
Temperature x Photoperiod			N.S.	P<0.01	N.S.	P<0.01
Oxygen x Photoperiod			P<0.01	N.S.	N.S.	N.S.
Temperature x Oxygen x Photoperiod			N.S.	N.S.	N.S.	N.S.

6. Summary of the relationship of erythrocytic electrolytes to hemoglobin (mmol. mmol Hb^{-1}). Reported as the mean ± standard error (95% confidence intervals)

Experimental Conditions			Measurements	
Temp. °C	Oxygen %	Light hr.	Cl^- : Hb	Mg^{+2} : Hb
20	25-35 (Hypoxia)	16L: 8D	24.0 ± 0.5 (22.8 - 25.1)	1.33 ± 0.05 (1.21 - 1.44)
20	25-35 (Hypoxia)	8L:16D	22.3 ± 1.0 (19.9 - 24.6)	1.35 ± 0.07 (1.18 - 1.51)
20	70-80 (Normoxia)	16L: 8D	26.0 ± 1.0 (23.7 - 28.2)	1.29 ± 0.05 (1.16 - 1.41)
20	70-80 (Normoxia)	8L:16D	21.8 ± 1.7 (18.0 - 25.5)	1.32 ± 0.05 (1.20 - 1.43)
5	25-35 (Hypoxia)	16L: 8D	17.5 ± 1.3 (14.5 - 20.4)	1.70 ± 0.11 (1.44 - 1.95)
5	25-35 (Hypoxia)	8L:16D	19.8 ± 0.9 (17.8 - 21.7)	1.49 ± 0.04 (1.39 - 1.58)
5	70-80 (Normoxia)	16L: 8D	16.8 ± 0.6 (15.4 - 18.1)	2.06 ± 0.07 (1.89 - 2.22)
5	70-80 (Normoxia)	8L:16D	17.3 ± 0.8 (15.4 - 19.1)	1.76 ± 0.06 (1.61 - 1.90)
Anova Table				
Temperature			P<0.01	P<0.01
Oxygen			N.S.	P<0.01
Photoperiod			N.S.	P<0.05
Temperature x Oxygen			N.S.	P<0.01
Temperature x Photoperiod			P<0.01	P<0.01
Oxygen x Photoperiod			N.S.	N.S.
Temperature x Oxygen x Photoperiod			N.S.	N.S.

reduced under these circumstances.

Oxygen

Differences in oxygen availability had no effect on chloride content. Plasma magnesium levels were, however, elevated to some extent under hypoxic conditions, with the converse being true of erythrocytic concentrations.

Photoperiod

Plasma, but not red cell composition, was affected by photoperiod. Chloride concentrations were somewhat lower on the 16L:8D as compared to 8L:16D regime, whereas the converse was true of plasma Mg^{+2} levels.

Significant interactions between temperature and oxygen were most obvious in relation to red cell composition, with a barely significant interaction also apparent in the case of plasma Cl^{-}. Temperature-photoperiod interactions were also highly significant in the case of cellular ion levels, but absent in the instance of plasma. Only one significant oxygen-photoperiod interaction was observed; again this involved plasma Cl^{-}.

As indicated in Table 5, animals exposed to monimal "summer" conditions (20^{o}C-hypoxic-16L:8D) were characterized by mean or significant elevations in plasma and red cell Cl^{-} concentrations and plasma Mg^{+2} levels by comparison with "winter" trout. By contrast, erythrocytic Mg^{+2} levels in "winter" fish were well above those observed in the group maintained under summer conditions. In the specific instance of plasma chloride, temperature and concentration were positively correlated, with the converse true of extended photoperiod. Oxygen availability had little effect. Consequently, the modest difference observed ("summer" - 124.4 ± 1.31, "winter" - 121.9 ± 1.91 $mmol\,lit^{-1}$) may reflect the outcome of opposing or neutral influences. Red cell Cl^{-} levels, on the other hand, were not affected by either oxygen availability or photoperiod. The higher concentrations seen in "summer" fish can then be attributed to temperature-induced processes. Increases in temperature and light duration, and reduced

oxygen availability were associated with elevation of plasma Mg^{+2} concentration. Thus, plasma Mg^{+2} levels of "summer" fish significantly exceeded those of "winter" animals. Similarly, the reduced erythrocytic Mg^{+2} content of "summer" fish also appears to be consistent with the effects of increased temperature and reduced oxygen availability. Photoperiod effects were not significant. In short, under the more realistic circumstances provided by an acclimatization rather than acclimation protocol, the influence of temperature tends, on the whole, to emerge as more effective than either oxygen availability or photoperiod in relation to ionic composition.

Table 6 also includes data for molar $[Cl^-]:[Hb]$ and $[Mg^{+2}]:[Hb]$ ratios (n.e., in calculation of these values a mean molecular weight of 64,500 was assumed for hemoglobin). The former displayed little variation in relation to photoperiod or oxygen availability, but increased sharply and significantly at higher temperatures. $[Mg^{+2}] : [Hb]$, on the other hand, proved to be sensitive to all factors. Significant decreases in ratio were associated with elevated temperature, hypoxia and reduced day length. In addition, significant interactions between temperature and both oxygen availability and photoperiod were evident. The significant elevation in $[Cl^-] : [Hb]$ in "summer" fish (24.0 ± 0.50) as compared to those maintained under "winter" conditions (17.3 ± 0.80) is clearly attributable to temperature, since only this factor had influence. The corresponding reduction in $[Mg^{+2}]:[Hb]$ ("winter" - 1.76 ± 0.06; "summer" - 1.33 ± 0.05) presumably reflects the effects of increased temperature and hypoxia. These presumably masked the opposing influence of extended day length.

DISCUSSION

The principal findings of the present study can be summarized as follows.

(1) Total hemoglobin and hematocrit increased with increase in temperature, exposure to hypoxia and reduction in light period. Significant interactions were observed between temperature and photoperiod and oxygen and photoperiod. However, under selected conditions, some of these effects could be masked. For example, comparison of animals under 'summer' and 'winter' conditions indicated that photoperiod effects were not expressed. Presumably, the effects of temperature *per se* and oxygen availability *per se* were stronger than those of photoperiod.

(2) Of the 12 hemoglobin components detected, 5, though consistently present, constituted only a minor element of the hemoglobin system. Furthermore, they exhibited little variation. This was also true of the most abundant fraction. The remaining 6 isomorphs, collectively from 66.1 to 71.6% of the total, exhibited considerable variation. Of the factors examined, photoperiod appeared to be the weakest in terms of effects on both actual and relative concentrations of isomorphs. Photoperiodic effects were also masked by those of temperature and oxygen availability. Oxygen availability appeared as the most effective factor influencing absolute amounts of these isomorphs.

(3) Concentrations of Cl^{-} and Mg^{+2}, critical ionic elements of the hemoglobin-oxygen affinity regulating system, also exhibited well-defined variation. Chloride ion levels in both plasma and red cells, for example, were increased at the higher temperature. This was also true of plasma Mg^{+2}, although the reverse was observed in the case of red cell Mg^{+2} content. Oxygen availability also had pronounced effects on Mg^{+2} levels and, again, an inverse relation was observed between plasma and erythrocytic Mg^{+2} levels. Oxygen availability had no effect

on [Cl^-] in either plasma or red cells. However, plasma Cl^- concentrations were reduced on the 16L:8D photoperiod regime as compared to 8L:16D. The converse was true of plasma Mg^{+2}. No photoperiodic influence on red cell Mg^{+2} or Cl^- levels was observed.

These findings will be discussed in turn.

Hemoglobin and hematocrit

Several studies have demonstrated increases in either hemoglobin or hematocrit or both with increases in environmental temperature in a variety of teleostean species (Table 7). Many of these have specifically considered rainbow trout (DeWilde and Houston, 1967; Houston and Cyr, 1974; Houston and Smeda, 1979). With increased temperature, which leads to increased metabolic oxygen requirements under circumstances of diminished oxygen availability, blood oxygen-carrying capacity is frequently enhanced by increasing red cell number; coupling this with reductions in cell volume - which increase oxygen diffusion rates - and modest increases in mean cellular hemoglobin content. Presumably such changes stem initially from release of erythrocytes from storage areas and, in the longer term, from increased erythropoietic activity. This can be regarded as advantageous, despite accompanying increases in blood viscosity and thus cardiac work requirements. Although energy must be expended in erythrocyte formation and hemoglobin synthesis, the cost of increasing oxygen-carrying capacity is presumably less than that entailed in operation of the cardiovascular and ventilatory systems at high, sustained rates. In general, present findings confirm those of several earlier studies in terms of thermoacclimatory changes in hemoglobin and hematocrit, and indicate that erythropoiesis is increased at higher temperatures. Chudzik and Houston (1983) provide direct evidence of this in goldfish. Although it has not yet been

Table 7: Thermoacclimatory changes in some hematological indices of two members of family-Salmonidae. Hb, hemoglobin, $g.dl^{-1}$; Hct, hematocrit, %; RBC, red cell count, $10^{-6}.mm^{-3}$; MEV, mean erythrocytic volume, μ^3; MEHbc, mean erythrocytic hemoglobin content, $\mu\mu g.\ cell^{-1}$.

Species	Acclimation Temperature °C	Hb	Hct	RBC	MEV	MEHbc	Reference
Salvelinus	2	7.2±0.2	34.1±0.9				Houston & DeWilde
fontinalis	5	8.3±0.2	37.6±0.5				(1968)
	8	8.5±0.2	36.8±0.6				
	10	7.1±0.2	35.2±0.8				
	20	7.6±0.2	37.2±1.1				
Salmo							
gairdneri	3 (summer)	6.4±0.8	29.0±2.1	1.09±0.08	259±22.1	22.2±1.7	DeWilde & Houston
	7	6.4±0.6	30.3±2.9	1.14±0.13	268±28.2	20.8±1.3	(1967)
	11	6.8±1.2	32.3±2.9	1.36±0.13	253±48.6	21.5±3.2	
	14	7.3±0.7	30.6±2.0	-	273±23.8	23.7±2.5	
	17	6.8±0.8	31.7±0.2	1.26±0.16	253±37.7	21.7±2.7	
	21	8.3±1.1	33.6±2.5	1.44±0.12	237±19.0	24.6±2.2	
	4 (winter)	6.5±0.6	28.4±2.9	1.19±0.14	241±18.6	22.1±1.4	
	7	7.3±0.6	29.7±1.5	1.25±0.12	239±18.8	24.3±1.6	
	11	8.2±0.5	34.5±1.7	1.44±0.10	240±11.5	23.8±1.4	
	14	8.2±1.4	33.4±3.8	1.30±0.14	252±15.8	24.9±1.7	
	17	8.0±0.5	32.4±1.4	1.40±0.09	232±12.8	24.7±1.6	
	18	8.3±0.9	34.5±2.0	-	-	23.4±1.7	
	21	8.5±0.8	34.7±2.6	1.51±0.11	232±23.1	24.2±0.9	

Table 7 (Cont'd.)

Species	Acclimation Temperature °C	Hb	Hct	RBC	MEV	MEHbc	Reference
Salmo	2	6.3±0.2	32.7±0.7				Houston & Cyr
gairdneri	10	7.3±0.1	42.9±0.5				(1974)
	18	8.3±0.02	46.6±0.7				
	2 (summer)	8.1±0.2	32.8±1.1	1.19±0.05	277±9.8		Houston & Smeda
	10	8.2±0.2	33.7±0.7	1.36±0.04	252±6.3		(1979)
	18	8.1±0.3	33.2±1.0	1.33±0.06	255±12.1		
	2 (winter)	8.1±0.3	31.5±0.9	1.32±0.05	241±6.7		
	10	8.2±0.3	31.6±1.1	1.23±0.06	263±8.6		
	18	7.6±0.4	30.8±1.6	1.28±0.05	242±6.5		
	2 (summer)		34.0				Murphy & Houston
	10		35.8				(unpub.)
	18		44.1				
	2 (winter)		32.1				
	10		34.6				
	18		41.7				

demonstrated, it is reasonable to hypothesize that erythropoietic activity is likely to be induced by hormonal stimuli (i.e., formation and release of an erythropoietin-like hormone; Zanjani et al, 1969) analogous to higher vertebrates.

Freshwater fishes are, however, inconsistent in this respect. Absence of temperature-related hematological response has been reported in some studies, including several on rainbow trout (Houston and Smeda, 1979). This was also the case with white sucker, Catostomus commersoni, carp, Cyprinus carpio, goldfish, Carassius auratus, carp-goldfish hybrid (Houston et al, 1976) and tench, Tinca tinca (Eddy, 1973). In addition, antiadaptive responses to temperature have also been reported (eg., reduction in hemoglobin content, hematocrit and/or red cell numbers) (Houston, 1980). These were observed in goldfish (Houston and Rupert, 1976), brown bullhead, Ictalurus nebulosus (Grigg, 1969) and pumpkinseed, Lepomis gibbosus (Houston et al 1976). The basis of this is not clear. However, seasonal variations in hematological status have been reported in several species including carp (Murachi, 1959; Houston and DeWilde, 1968), rainbow trout (DeWilde and Houston, 1967; Denton and Yousef, 1975; Houston and Smeda, 1979), Pseudopleuronectes americanus (Umminger and Mahoney, 1972; Bridges et al, 1976). Houston et al (1976) have hypothesized that temperature may not, by itself, prompt erythropoiesis unless accompanied by other seasonally-appropriate conditions. This infers that synergism between stimulating factors exists. The present study provides evidence supportive of this contention in terms of the interactions identified.

Hematological responses to hypoxia have also been examined (Table 8) in channel catfish, Ictalurus punctatus (Scott and Rogers, 1981), rainbow trout (Holeton and Randall, 1967; Swift and Lloyd, 1974) and

killifish, Fundulus heteroclitus (Greaney and Powers, 1978). In the present study, highly significant increases in hemoglobin and hematocrit were associated with exposure to hypoxia. Hematocrit levels increase as a consequence of exposure to oxygen-deficient conditions (Holeton and Randall, 1967; Wood and Johansen, 1972; Swift and Lloyd, 1974).

It is commonly assumed that the stimulating effects of these variables are mediated by erythropoietin or some erythropoietin-like hormone. In mammals, erythropoietin, a glycoprotein hormone which is released from the kidney, stimulates erythropoiesis and leads to increases in hematocrit. Gordon (1959) proposed a model for mammalian production of the erythropoietin. Hypoxia induces the kidneys to release a renal erythropoietic factor (REF) into the plasma. This REF acts on a plasma protein to generate active erythropoietin (ESF, or erythropoiesis-stimulating factor). ESF then acts on erythropoietin-responsive cells (ER) in blood-forming areas causing initiation of erythropoiesis (Figure 14).

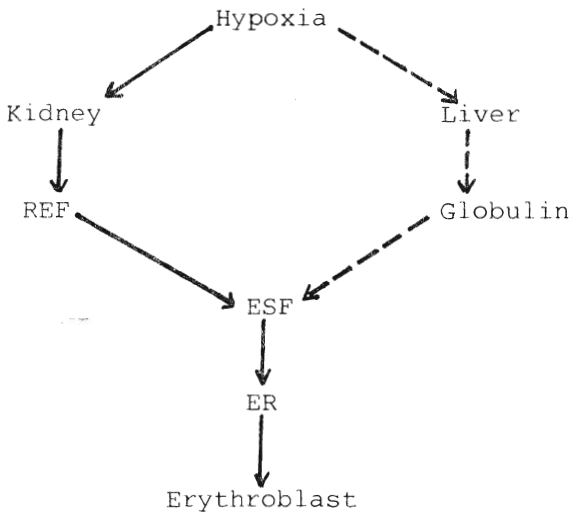


Figure 14: Flow diagram illustrating mechanism of erythropoiesis (from Gordon and Zanjani, 1971)

Interestingly, Gordon and Zanjani (1971) were unable to detect erythropoietic activity in carp serum. It should be noted, however,

Table 8: Hypoxic-associated changes in hematological parameters in representative freshwater fishes . Hb, hemoglobin, $g.dl^{-1}$; HCT, hematocrit, %; RBC, red cell count, 10^{6} $-\mu l$; MEV, mean erythrocytic volume, μ^{3}; MEHbc, mean erythrocytic hemoglobin content, picograms; MCHC, mean corpuscular hemoglobin concentration, $picograms.\mu^{-3}$.

* originally shown in the unit of $mmol\ tetramer.lit^{-1}$

¶ MEHbc in percentage unit

Species	O_2 level	Hb	HCT	RBC	MEV	MEHbc	MCHC	Reference
Ictalurus	7.5 ppm	7.74	34.60	2.18	159.8	36.50	0.234	Scott & Rogers
punctatus	1.5 ppm	10.42	32.60	2.33	144.9	46.37	0.325	(1981)
Anguilla	140 mmHg	4.9	23.4					Wood & Johansen
anguilla	15 mmHg	7.3	36.0					(1972)
Cyprinus	120-130 mmHg		26.4	0.94				Weber & Lykkeboe
carpio	30 mmHg		29.0	1.02				(1978)
Fundulus	8.4-9.0 ppm		23±1.5					Greaney & Powers
heteroclitus	1.5-2.5 ppm		36±1.0					(1978)
Salmo	control (?)	4.56±0.58	33±4	0.949±0.096	348±42	14.1±2.6 ¶		Swift & Lloyd
gairdneri	4.5mgO_2.lit	5.66±0.29	40±6	1.217±0.216	344±60	13.3±2.6 ¶		(1974)
	150 mmHg	6.45 *	28.2					Tetens & Lykkeboe
	50 mmHg	7.22 *	35.3					(1981)
Oncorhynchus	7.9 ppm		33.5					Giles & Vanstone
kisutch	5.8 ppm		34.4					(1976)
(pre-smolt)	3.1 ppm		39.3					
Lagodon	ambient (?)	8.38±0.72	32.0±3					Cameron
rhomboides	1.30mg.lit^{-1}	8.98±0.81	41.4±4.8					(1970)

that their assays for erythropoietic activity involved the use of mice. Gordon and Zanjani speculate that failure to detect erythropoiesis, in this case, may have been due to species differences in the hormone. This infers that while vertebrate erythropoietin may possess structural similarities, differences in chemical groups may well underlie group specificities (Gordon and Zanjani, 1971). Erythropoietin was, however, detected in the plasma of blue gourami, Trichogaster trichopterus (Zanjani et al, 1969). Temperature-induced increases in erythropoietin levels have not yet been reported. It is, however, reasonable to anticipate that if similar mechanisms operate in fishes, the increases in hemoglobin and hematocrit levels with elevated temperature and reduced oxygen will ultimately be linked to changes in the level of this hormone. However, erythropoietin may not be the only, or even the principal hormonal agent stimulating increase in red cell formation and hemoglobin synthesis. Under unfavourable conditions (eg., high temperature, acute hypoxia) catecholamine levels increase sharply (Butler et al, 1978, 1979). In vitro studies by J. E. Keen in this laboratory indicate that catecholamines also stimulates hemoglobin synthesis in erythrocytes.

Reduced photoperiod leads to pronounced and significant increases in both hemoglobin and hematocrit values. This contrasts with the findings of Murphy and Houston (unpublished data) who noted mean, but not statistically-significant increases in the hematocrits of trout acclimated to longer day lengths. The basis of this discrepancy is not clear. However, it may be noted that these authors did not manipulate O_2 availability.

In conclusion, at high temperature, under hypoxic conditions and with abbreviated day lengths blood oxygen carrying capacity increases. This is probably a consequence of increased erythropoiesis, due, in part at least, to hormones such as erythropoietin-like hormone or catecholamine, and/or the liberation of red cells from storage sites. Elevated hematocrit values

may be the result of increased red cell number which, in turn, would also be a consequence of activated erythropoiesis and release of stored erythrocytes and/or increased mean corpuscular volume (MCV). Although increased hematocrit may be regarded as a suitable adaptation, it may result in increased blood viscosity leading to higher metabolic energy demands in the circulatory system. However, very recently, by using nuclepore filters, Hughes and Kikuchi (1984) and Hughes et al (1982) have indicated an increase in red cell deformability which effectively reduces the resistance to blood flow through the gills and other parts of microcirculation in hypoxic rainbow trout and in 37°C-acclimated yellowtail, Seriola quinqueradiata respectively.

In summary, the data obtained in this study support the hypothesis that the environmental factors considered influence blood-oxygen carrying capacity.

Organizational Changes in the Hemoglobin System

Variations in temperature, oxygen and photoperiod also affected individual isomorphs. As stated earlier, the twelve hemoglobin mobilities detected could be categorized in terms of relative abundancies as major, intermediate and minor components. The relative abundancy of the most abundant of these was not influenced by any of the factors considered. Its absolute concentration was, however, increased at 20°C. The second most abundant fraction, A3, displayed no temperature-related influence on absolute concentration, although its relative abundancy was decreased by increased temperature. Among the intermediate components, C3 and A1 clearly indicated the increases in their actual amounts. Briefly, of all major and intermediate electrophoretic components, the actual compositions of C4, C3 and A1 was increased, although A3 did not alter with temperature.

Earlier studies on this species using acrylamide gel disc electrophoresis lead to the separation of 9 mobilities (Houston and Cyr, 1974). Of these, one major component, T2 (22-28%) and one intermediate fraction, T4 (13-17%) increased in actual and relative concentrations with increases in temperature. Increase in actual content with decrease in percentage abundancy was also observed in the case of another major fraction, T7 (22-26%). Despite the difference in the electrophoresis method used, similarities between this, and the present study can be seen. For example, the pattern of variation in actual contents of T2 and T4 with temperature is quite similar to those of C4, C3 and A1 in the present study and in terms of relative abundancy, T7 is comparable to A3.

Other teleosts also exhibit temperature-related changes in the relative abundancies of specific hemoglobin polymorphs. In the relatively eutythermal white sucker, Catostomus commersoni, the concentrations of all major components increased at high temperatures (Houston, 1980). Carp, which are among the most eurythermal of the teleosts studied in

this laboratory, displayed variation in all hemoglobin polymorphs. For example, one major component (35-41%) and an intermediate fraction (19-26%) increased their abundancies with thermal acclimation, although another major fraction (34-46%) decreased (Houston, 1980).

The foregoing studies revealed quantitative, but not qualitative responses, i.e., gain or loss of isomorphs. However, goldfish (Houston and Cyr, 1974; Houston et al, 1976) appear to adapt both qualitatively and quantitatively to temperature changes. Falkner and Houston (1966), Houston and Cyr (1974), Houston and Rupert (1976) and Houston et al (1976) reported that goldfish acclimated to cold temperature (<10°C) normally had two components, whereas warm-acclimated fish possessed three hemoglobins. Houston and Rupert (1976) believed that this was the result of the aggregation of preexisting subunits rather than activation of an additional globin gene.

Based on previous studies, Houston (1980) concluded that:

(1) The teleostean hemoglobin system possesses a considerable degree of thermolability.

(2) Interspecific differences exist.

(3) Increased heat tolerance is correlated with reduced hemoglobin system complexity (Table 9).

Weber et al (1976b) commented upon differences between geographically-separated populations of the same species in their adaptation to environmental temperature or other factors. They point, for example, to differences between the hemoglobins of rainbow trout populations from Buffalo, N. Y., Italy and Denmark. The first one was characterized by a larger number of cathodally-migrating hemoglobins, less sensitive to temperature, pH and organophosphates than those of the European populations. They regarded this as indicative of intraspecific adaptation to localized environmental conditions. The population of trout used in the present study closely resembled that from Buffalo in terms

Table 9: Changes in hemoglobin complex in relation to thermal tolerance (Houston, 1980)

Species	Upper L.T* °C	No. of Hbs
Oncorhynchus keta	23.6	14
O. gorbuscha	23.9	16
O. nerka	23.8	9-19
O. kisutch	25.0	11-19
O. tshawytscha	25.1	18
Salmo gairdneri	25.5	7-11
Salvelinus fontinalis	26.6	13-15
Catostomus commersoni	31.2	8
Lepomis gibbosus	35.5	6
L. macrochirus		6
Ictalurus nebulosus	36.0	7
Cyprinus carpio	35.7	3-4
Carassius auratus	38.6	2-3
Fundulus heteroclitus	31.3	4

* L.T = Lethal temperature

of the relatively larger number of cathodal hemoglobins present.

It has been known for several years that the hemoglobin isomorphs of fishes can differ in their physiological properties. Brunori (1975), for example, grouped the hemoglobins of rainbow trout into two categories; those which have little or no sensitivity to temperature, pH and organophosphates, and those whose oxygen affinity is influenced by these factors. Brunori (1975) and Weber et al (1976b) carried out the electrophoretic separation of trout hemoglobins on chromatographic columns and purified each fraction. The properties of each fraction were then examined (Table 10). Since hemoglobin isomorphs are more or less different in their properties, it has been hypothesized that quantitative and qualitative variations represent selective modifications favouring components suited to particular environmental conditions. From Table 10, it can be seen that the oxygen affinity of cathodal components are insensitive to temperature, pH and organophosphate; the reverse is true of anodal groups. Interestingly, low temperature sensitivity of cathodal fractions are consistent in other teleosts (Table 11).

Table 10: Properties of rainbow trout hemoglobin isomorphs.

(Brunori, 1975; Weber et al, 1976b)

Properties	Hb I	II	III	IV	V	VI
Proportion (%)	15-20	15-20	3	60-70		
Isoelectric point (pH)	8.6	7.4	6.7	6.2-6.5	6.1	5.4
Stability const. ($K_4,2$)*	7.5×10^{-8}	-	-	5.2×10^{-8}	-	-
O_2-affinity (at pH7; 20 °C)	high	-	-	low	-	-
ATP sensitivity	absent	present	present	present	present	present
Bohr effect	absent (reversed)	absent	present	present	-	-
Root effect	absent	absent	-	present	-	-
Temperature sensitivity ΔH (Kcal.mol^{-1})	low (-3)	low (-4)	low	high (-14.5)	high	high

Increased relative mobility to anode ⟶

* $Hb_4 \overset{K_4,2}{\rightleftharpoons} 2\ Hb_2$

Table 11: Comparison of thermalsensitivity of hemoglobin isomorphs observed in some teleosts.

ΔH (Van't Hoff enthalpy) = 2.303 R. Δ log P50 / Δ (I/T) Kcal.mol^{-1}

Fish	ΔH (cathodal Hbs)	ΔH (anodal Hbs)	References
Oncorhynchus keta	-2.4	-7.2	Hashimoto & Matsuura (1960)
Anguilla japonica	-7	-13	Yamaguchi et al (1962)
A. anguilla	-13.3	-16.0	Weber et al (1976a)
Salmo gairdneri	-1.0	-8.5	Weber et al (1976b)

Hence, Weber et al (1976b) concluded that the cathodal hemoglobins in trout will tend to stabilize the temperature-induced variations in oxygen affinity of whole blood.

Studies by Binotti et al (1971), Brunori (1975), and Weber et al (1976b) investigating the physiological properties of trout hemoglobin isomorphs were carried out by column chromatography. No comparable studies have been carried out on the properties of isomorphs separated by cellulose acetate electrophoresis. However, patterns of variation in isomorph abundancies can be used to establish at least approximate correspondences of principal components. In the present study, for example, the two most abundant cathodal components increased in actual concentration at the higher temperature. No significant change in the concentration of the major anodal fraction, A3, was evident. Since the former became abundant at high temperature, it might be postulated that these isomorphs may possess certain properties which would be physiologically advantageous in this unfavourable situation. From this assumption it can be hypothesized that oxygen affinity of C4 and C3 (probably other cathodal fractions too) may be independent

of temperature and pH. In other words, they are likely to be similar to Brunori's cathodal components and thus, increases in the abundancies of such isomorphs will be the efficient response to higher temperatures stabilizing oxygen supply to tissue. On the other hand, the relative concentration of A3, which is the most abundant fraction within the anodal group did not alter at high temperature so that it can be postulated that like Brunori's anodal Hb IV, oxygen affinity of A3 may be thermally and pH-sensitive.

A few studies have examined hemoglobin system changes during adaptation to hypoxic environments. Weber and Lykkeboe (1978), for example, reported that the relative abundancies of hemoglobins from carp exposed to hypoxic- and normoxic conditions were not significantly altered. Similar observations on eel, Anguilla anguilla were reported by Weber et al (1975). Much the same was true of the relative abundancies of trout hemoglobins. In the present study, hypoxia lead to elevated actual abundancies of all major and intermediate isomorphs except A1. It was apparent that oxygen availability was more important than temperature.

It has been demonstrated that hypoxic acclimation is associated with increased pH and decreased organophosphate content in erythrocytes (Soivio and Nikinmaa, 1981). Anodal isomorphs whose oxygen affinity is, according to Brunori (1975), largely dependent on these modulators, would likely to be saturated with oxygen. It can be suggested that trout may adapt to hypoxic environments by increasing the contents of anodal isomorphs as well as that of the cathodal group whose oxygen affinity is believed to be independent of these modulators. In short, to enhance the blood-oxygen carrying capacity under hypoxic conditions both cathodal and anodal fraction abundancies may increase. This hypothesis is supported by the present findings in which almost all cathodal and anodal isomorphs were significantly elevated in abundancies following exposure to hypoxia.

Reports on photoperiod effect are not available so far. This factor

appeared to have little real influence on the hemoglobin system, since no distinct changes in relative abundancies were detected. The actual contents of A3 and three cathodal hemoglobins, C4, C6 and C7 were augmented at short day length. However, this effect seemed to be masked by temperature or oxygen or both when 'summer' and 'winter' trouts were compared (Fig. 13). It is apparent that both temperature and hypoxia tended to increase these hemoglobin components strongly and thus, masked the effect of photoperiod.

It is not yet known, however, how hemoglobin isomorph abundancies are regulated; although synthesis or reaggregation represent obvious possibilities.

In conclusion, it can be postulated that teleosts like rainbow trout inhabiting cold, well-aerated water, may respond to high temperatures by increasing the abundancies of at least some cathodal isomorphs whose oxygen affinity is, presumably, independent of modulators such as temperature, pH and organophosphates. In other words, the cathodal group may act as an important and major oxygen supplier in such a thermally unfavourable situation. The reverse would be true of the anodal group. In this way, trout may stabilize the variations in oxygen affinity of whole blood at high temperatures. Under hypoxia, almost all hemoglobin components increased in abundancies and appeared to enhance oxygen-carrying capacity. It has been clearly observed in previous studies that hypoxic acclimation is followed by increased pH and decreased organophosphate contents in trout red cells. Hence, the increased anodal components whose oxygen affinity is believed to be sensitive to temperature, pH and organophosphates are not subject to counteracting effects of these oxygen-affinity modulators. No significant light influence on the multiple-hemoglobin system was observed and even seemed to be masked by temperature and oxygen.

Plasma ion composition:

High temperatures are associated with increases in ventilatory flow, cardiac output and effective branchial surface area, and lead to increased water influx and electrolyte efflux (Randall et al, 1972). Osmoregulatory response includes decrease in water permeability, decreases in Na^+ and Cl^- permeabilities, increased activity of Na^+ and Cl^- uptake mechanisms and increased reabsorption of Na^+ and Cl^- from the urine (Maetz, 1974).

Branchial electrolyte transfers involve two principal processes: exchange diffusion and active transport. The activities of these systems are temperature-dependent (Maetz, 1972; Cameron, 1976). Their activation at higher temperature is believed to be responsible for compensation of ion losses in teleosts including rainbow trout (Houston and McCarty, 1978; Houston and Koss, 1984), goldfish (Murphy and Houston, 1974) and carp (Houston et al, 1968). These studies exhibit very little change in plasma ion concentrations over broad intermediate ranges of temperature. Of the two plasma ions considered in the present study, Cl^- was largely unaffected by oxygen although it tended to increase at 20°C and on the shorter day-length. However, the average percentage changes were small (6.3% and 5.8% respectively). Few reports deal with plasma Mg^{+2} regulation in fishes. However, it was noted in this study that oxygen and photoperiod has less impact on plasma Mg^{+2} concentration than did temperature. The mean percentage changes in relation to oxygen availability and photoperiod were only 7.1% and 9.0% respectively, whereas in the case of temperature a mean change of 21.5% was observed. The mechanisms which might account for this are not known.

Stresses such as temperature changes, acute hypoxia, handling and exercise stimulate the production of catecholamines, particularly adrenaline, in many fishes including elasmobranchs (Nakano and Tomlinson, 1967;

Butler et al, 1978). Catecholamines affect ionic regulation, increasing branchial water permeability (Pic et al, 1974) and prompting endosmosis in freshwater teleosts. Demineralization also occurs (Pic et al, 1975); presumably, in part at least, as a consequence of the inhibition of ATPases. Na^+ and Cl^- uptake through gills are then reduced (Specker and Schreck, 1980). These hormones also affect branchial circulation (Jones and Randall, 1978), increasing secondary lamellae perfusion (Keys and Bateman, 1932) but reducing blood supply to "chloride" cells or ionocytes which are important in ion regulation. Although stress-induced elevation of catecholamine levels tend to prompt osmoregulatory imbalances in freshwater fishes, other hormones may play a compensatory role. McKeown and Peter (1976), for example, pointed out that plasma and pituitary prolactin levels varied with temperature and photoperiod. The primary function of this hormone lies in restriction of passive ion efflux (Maetz, 1972; Cameron, 1976). Moreover, photoperiod or thermal activation of the thyroid (Eales, 1965) also stimulates branchial Na^+/NH_4^+ exchange maintaining Na^+ balance. These factors not only increase production of prolactin and thyroid hormones, but also that of cortisol (Fryer, 1975) which stimulates the activity of ATPase. Thus, some compensation for the effects of high temperature and extended photoperiod would be expected, and this is borne out by the studies previously cited.

Plasma Mg^{+2} levels were significantly augmented in nominal "summer fish". Among the factors considered, temperature appeared to be the most effective. For example, significantly higher plasma Mg^{+2} levels were encountered at 20°C. Furthermore, those associated with hypoxia and prolonged day length were less pronounced. Few studies have considered Mg^{+2} regulation in teleosts. However, increase in plasma Mg^{+2} at higher temperatures have been reported in rainbow trout (Murphy, 1978; Houston and Smeda, 1979; Houston and Koss, 1984a,b). We are aware of no studies specifically considering the effects of hypoxia and photoperiod. It may

be noted that all conditions associated with increase in plasma Mg^{+2} were 'summer' characteristics.

Erythrocytic ion composition:

Cold-acclimated animals were characterized by distinctly higher red cell Mg^{+2} levels than those held at 20°C. Houston and Koss (1984 a,b) have reported similar results, although the basis of this is not clear. Gunther et al (1984) observed that Mg^{+2}-loading of avian erythrocytes lead to net Mg^{+2} efflux through activation of Mg^{+2}-specific membrane channels, until the original free Mg^{+2} content was restored. It is not known whether similar mechanisms exist in teleostean red cells. We are aware of no species with compelling evidence of active Mg^{+2} uptake by red cells. Although mechanisms generally governing cellular Mg^{+2} levels are not clear, the significance of the changes observed can be inferred. The affinity of Mg^{+2} for organophosphates is extremely high and increases with temperature (Bunn et al, 1971). Adjustments in erythrocytic Mg^{+2} content therefore provides a potent means for adjusting nucleoside triphosphate availability for interaction with hemoglobin. Since these are critical modulators of hemoglobin-oxygen affinity, Mg^{+2} shifts can lead to changes in the readiness with which oxygen is taken up and released. Lower levels of Mg^{+2} are associated with increases in "free" organophosphates which can then bind to hemoglobin. In this way a reduction in red cell Mg^{+2} content can prompt decreases in Hb-O_2 affinity (Bunn et al, 1971; Gillen and Riggs, 1971; Weber and Lykkeboe, 1978). The present study demonstrated significant decreases in Mg^{+2} level at high temperature. Given that NTP levels are not changed, this would be expected to decrease Hb-O_2 affinity and facilitate oxygen release to tissue. Dobson and Baldwin (1982) have recently demonstrated that this, in fact, occurs with exposure of the Australian blackfish, Gadopsis marmoratus, an active species living in well-oxygenated water to higher temperatures. Heath and Hughes (1973) also

examined the response of rainbow trout when water temperature rapidly increased from 14° to 27°C and provided evidence consistent with thermal reduction of Hb-O_2 affinity. Recently, Houston (1984) reported that over a broad range of low to intermediate constant temperatures, the Mg^{+2} levels of representative eurythermal and stenothermal species, including the rainbow trout, did not vary. However, further increases in water temperature were associated with reductions in Mg^{+2} content.

Although Cl^- levels were not influenced by hypoxia and photoperiod, exposure to high temperatures lead to significant increases in both red cell Cl^- content and $[Cl^-]:[Hb]$. Similar findings have been reported by Smeda (1979) and Houston and Koss (1984a). It was demonstrated that ion transport enzyme activities are involved in temperature-related variations in erythrocytic Cl^- concentration. The increased activity of carbonic anhydrase at high temperatures may be related to the red cell CO_2 hydration, and its export from the red cell as bicarbonate in exchange for Cl^-, resulting in higher cellular Cl^- content (Fortes, 1977). The involvement of HCO_3-stimulated ATPase is less certain, but it may participate in enhancing Cl^- content in red cells (Houston, 1980).

The effect of Cl^- has not been examined in trout, but may be inferred from mammalian studies as noted in the Review of Letriture. Chloride affects mammalian Hb-O_2 affinity by favouring the deoxystate (Laver *et al*, 1977). If this is true of fish as well, Hb-O_2 affinity should decrease at high temperatures because of changes in erythrocytic Cl^- and Mg^{+2}. Interestingly, these effects are comparable to those of increased temperatures *per se*, and to the decreases in cell pH which are associated with increased temperature (Reeves, 1977). Thus, all factors favour the tense or deoxyhemoglobin state at high water temperature. This can be regarded as a significant adaptation in teleosts inhabiting well-oxygenated waters, i.e., those for which oxygen uptake is not a problem. Decreased Hb-O_2 affinity facilitates oxygen release to tissues under conditions in which metabolism

has been elevated by temperature.

Such responses might, however, be inappropriate for particular species. For example, the bullhead, Ictalurus nebulosa (Grigg, 1969) and killifish (Greaney and Powers, 1977) which occupy continuously or episodicaly hypoxic waters exhibit increased affinities when exposed to higher temperatures. They can therefore maximize use of what little oxygen is available and such species are, in a sense, oxygen scavengers.

Wood and Johansen (1972) investigated the response of eel to hypoxia and noticed decreased organophosphate levels and increased Hb-O_2 affinity. This is consistent with the findings of Weber et al (1975), Greaney and Powers (1978) and Weber and Lykkeboe (1978). Conclusions regarding the effects of altered Mg^{+2} are, of course, tentative in the absence of data on cellular organophosphate levels for it is the $[Mg^{+2}]$: [ATP] or $[Mg^{+2}]$: [GTP] relationship rather than Mg^{+2} per se which is of functional importance. In fact, there is some change in these (Weber and Lykkeboe, 1978; Nikinmaa and Weber, 1984) and in general these suggest that changes in $[Mg^{+2}]$: [NTP] will supplement those in [NTP] : [Hb]. Furthermore, because Mg^{+2} levels, like those of other ions, can be shifted relatively rapidly, variations in $[Mg^{+2}]$: [ATP] can be achieved more quickly than would be the case if NTP only were changed.

Briefly, oxygen carrying capacity would likely be increased at high temperature and in hypoxic conditions by increasing the amounts of hemoglobin and hematocrit. Chloride ion-reduced lowering of hemoglobin-oxygen affinity at high temperature acclimation was followed. As a consequence of the combination of these responses, nominal 'summer trout' appeared to be characterized by increased oxygen carrying capacity and decreased hemoglobin-oxygen affinity as well as increased ventilation to aid oxygen uptake. This is an advantageous response since more oxygen could be loaded at the gills and oxygen would be released easily at tissue level.

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CONCLUSIONS

(1) Blood-oxygen carrying capacity is enhanced with increased temperature, exposure to hypoxia and reduction in light period by increasing total hemoglobin and hematocrit.

(2) The twelve hemoglobin components are observed. The most abundant two cathodal isomorphs increase in their abundancies but the most abundant anodal isomorph does not alter with temperature. Hypoxic acclimation is associated with increases in contents of almost all isomorphs.

(3) Plasma Cl^- is largely unaffected by oxygen and it tends to increase at high temperature and on the shorter day length, but the average changes are small. Oxygen and photoperiod have less impact on plasma Mg^{+2} concentration than does temperature.

(4) Exposure to high temperatures lead to significant increase in red cell Cl^- but decrease in Mg^{+2} content. Although Cl^- is not influenced by oxygen, Mg^{+2} content decreases under hypoxic condition. Photoperiod has no effect on red cell ion levels.

(5) Nominal "summer" and "winter" trout are compared. It is assumed that "summer" trout is characterized by increased blood-oxygen carrying capacity and decreased Hb-O_2 affinity.

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APPENDICES

Appendix 1: Weight (Wt.,g), total length (T.L, cm), fork length (FL,cm), blood hemoglobin content (Hb,g.dl^{-1}), hematocrit (Hct,%), mean corpuscular hemoglobin concentration (MCHC,g.dl^{-1}), plasma Cl^{-} and Mg^{+2} (pCl^{-} and pMg^{+2}, mmol.lit^{-1}) and red cell Cl^{-} and Mg^{+2} level (rbc Cl^{-} and rbc Mg^{+2}, mmol.lit^{-1}) at 20°C, Normoxia, 16L:8D

Fish	Wt.	T.L	F.L	Hb	Hct	MCHC	pCl^{-}	rbc Cl^{-}	pMg^{+2}	rbc Mg^{+2}
1	69.8	19.8	19.0	7.6	38.8	19.58	106.4	84.6	1.15	2.95
2	73.1	20.8	19.9	8.1	37.7	21.48	115.6	74.0	0.94	4.20
3	86.3	21.6	20.5	9.8	48.2	20.33	126.3	84.4	1.07	4.97
4	75.9	20.2	19.5	8.7	38.9	22.36	130.2	75.7	0.88	3.97
5	56.5	17.6	17.1	4.5	23.0	19.56	113.0	84.0	0.86	4.24
6	54.8	17.7	17.4	7.0	37.8	18.51	140.6	90.9	0.92	4.23
7	70.0	18.0	17.6	7.0	32.7	21.40	129.1	72.1	0.92	4.23
8	56.7	18.2	17.9	6.5	30.0	21.66	123.8	86.7	0.80	4.76
9	53.7	17.6	17.0	5.5	24.5	22.44	130.2	87.8	0.88	5.03
10	83.1	21.8	20.8	6.5	28.4	22.88	125.0	77.9	0.80	4.24
11	46.3	17.6	17.1	5.5	25.4	21.65	140.4	94.6	1.15	4.23
12	50.0	19.9	17.7	4.6	23.7	18.98	115.8	89.8	0.94	3.16
$\bar{X}$	64.6	19.2	18.4	6.7	32.4	20.90	124.7	83.5	0.94	4.18
Sn-1	13.3488	1.6316	1.4035	1.6153	7.9051	1.4588	10.5158	7.1417	0.1200	0.6248
S^2	178.190	2.6624	1.9699	2.6093	62.4911	2.1283	110.5836	51.0044	0.0144	0.3904
$S\bar{x}$	3.8534	0.4710	0.4051	0.4663	2.2820	0.4211	3.0356	2.0616	0.0346	0.1803
U	73.1	20.3	19.4	7.8	37.4	21.83	131.3	88.0	1.01	4.58
L	56.1	18.2	17.6	5.7	27.4	19.97	118.0	79.0	0.86	3.78

Appendix 2: Weight (Wt.,g), total length (T.L, cm), fork length (FL,cm), blood hemoglobin content (Hb,g.dl^{-1}), hematocrit (Hct,%), mean corpuscular hemoglobin concentration (MCHC,g.dl^{-1}), plasma Cl^- and Mg^{+2} (pCl^- and pMg^{+2}, mmol.lit^{-1}) and red cell Cl^- and Mg^{+2} level (rbc Cl^- and rbc Mg^{+2}, mmol.lit^{-1}) at 20°C, Normoxia, 8L:16D

Fish	Wt.	T.L	F.L	Hb	Hct	MCHC	pCl^-	rbc Cl^-	pMg^{+2}	rbc Mg^{+2}
13	69.8	20.9	20.0	7.4	39.4	18.78	133.3	79.0	1.01	4.06
14	64.5	20.5	19.8	7.9	46.6	16.95	149.1	79.2	0.86	3.30
15	80.6	21.7	20.2	10.0	49.4	20.24	137.6	79.6	0.88	3.90
16	107.7	21.8	20.9	8.1	40.6	19.95	108.3	93.3	0.78	2.96
17	98.9	21.8	20.8	9.3	27.5	33.81	142.9	64.2	0.87	5.50
18	96.8	22.1	21.5	5.3	23.7	23.20	127.7	77.5	0.86	4.91
19	79.2	19.5	19.0	7.3	30.4	24.01	132.9	72.0	1.15	5.50
20	65.0	19.8	18.7	6.8	26.0	26.15	123.0	66.8	0.99	4.82
21	90.3	21.2	20.7	6.2	23.5	26.38	118.4	80.5	0.89	5.93
22	75.1	20.2	19.5	7.3	28.2	25.88	128.3	65.2	0.95	5.76
23	72.5	19.9	19.0	8.2	35.0	23.42	122.4	66.8	0.95	5.51
24	97.5	21.2	20.6	8.8	39.3	22.39	115.7	78.6	1.01	4.91
$\bar{X}$	83.1	20.9	20.1	7.7	34.1	23.43	128.3	75.2	0.93	4.75
Sn-1	14.5964	0.8891	0.8805	1.2723	8.8764	4.4437	11.6715	8.4997	0.0979	0.9851
S^2	213.0548	0.7906	0.7753	1.6187	78.7915	19.7471	136.225	72.2456	0.0095	0.9705
$S\bar{x}$	4.2136	0.2566	0.2541	0.3672	2.5624	1.2828	3.3692	2.4536	0.0282	0.2843
U	92.4	21.44	20.6	8.5	39.7	26.25	135.7	80.6	0.99	5.38
L	73.8	20.31	19.5	6.9	28.4	20.60	120.8	69.8	0.87	4.13

Appendix 3: Weight (Wt.,g), total length (T.L, cm), fork length (FL,cm), blood hemoglobin content (Hb,g.dl^{-1}), hematocrit (Hct,%), mean corpuscular hemoglobin concentration (MCHC,g.dl^{-1}), plasma Cl^- and Mg^{+2} (pCl^- and pMg^{+2}, mmol.lit^{-1}) and red cell Cl^- and Mg^{+2} level (rbc Cl^- and rbc Mg^{+2}, mmol.lit^{-1}) at 20°C, Hypoxia, 16L:8D

Fish	Wt.	T.L	F.L	Hb	Hct	MCHC	pcl^-	rbc Cl^-	pMg^{+2}	rbc Mg^{+2}
25	41.9	18.7	18.2	7,5	38.6	19.43	128.2	71.1	1.05	4.65
26	79.3	22.5	21.9	6.7	36.4	18.40	116.1	67.2	0.88	4.12
27	89.0	21.6	20.6	7.7	34.7	22.19	129.2	84.2	1.05	3.58
28	61.0	19.7	19.0	8.8	41.7	21.10	121.5	73.3	1.00	3.58
29	79.4	20.8	20.0	6.0	28.9	20.76	129.9	72.4	0.94	4.71
30	53.8	18.4	17.8	6.7	31.8	21.07	119.5	74.7	0.98	3.71
31	57.7	19.9	19.2	8.8	42.3	20.80	131.2	82.0	1.18	4.75
32	80.2	22.5	22.0	9.3	44.8	20.75	121.5	70.5	1.17	4.43
33	100.0	22.0	21.6	6.7	35.4	18.92	123.2	82.2	1.35	3.67
34	43.2	17.4	17.0	6.0	30.8	19.48	124.0	74.9	1.03	3.57
35	78.7	19.3	18.7	7.3	35.2	20.73	124.3	75.2	1.03	4.50
36	80.1	21.3	20.7	7.5	37.8	19.84	125.1	75.5	1.15	4.59
$\bar{X}$	70.3	20.3	19.7	7.4	36.5	20.28	124.4	75.2	1.06	4.15
Sn-1	18.3863	1.6946	1.6657	1.0886	4.8066	1.0790	4.5299	5.1431	0.1267	0.4980
S^2	338.0560	2.8717	2.7747	1.1851	23.1042	1.1643	20.5202	26.4515	0.0160	0.2480
$S\bar{x}$	5.3076	0.4891	0.4808	0.3142	1.3875	0.3115	1.3076	1.4846	0.0422	0.1660
U	81.9	21.4	20.8	8.1	39.5	20.97	127.3	78.5	1.15	4.51
L	58.6	19.3	18.7	6.7	33.4	19.60	121.5	71.9	0.96	3.78

Appendix 4: Weight (Wt.,g), total length (T.L, cm), fork length (FL,cm), blood hemoglobin content (Hb,g.dl^{-1}), hematocrit (Hct,%), mean corpuscular hemoglobin concentration (MCHC,g.dl^{-1}), plasma Cl^- and Mg^{+2} (pCl^- and pMg^{+2}, mmol.lit^{-1}) and red cell Cl^- and Mg^{+2} level (rbc Cl^- and rbc Mg^{+2}, mmol.lit^{-1}) at 20°C, Hypoxia, 8L:16D

Fish	Wt.	T.L	F.L	Hb	Hct	MCHC	pCl^-	rbc Cl^-	pMg^{+2}	rbc Mg^{+2}
37	88.8	21.6	21.2	9.5	41.5	22.89	134.3	71.5	1.04	4.21
38	63.9	21.1	20.2	9.5	42.8	22.19	134.9	70.1	0.96	3.62
39	62.4	18.9	18.5	14.6	60.9	23.97	130.5	70.9	1.0	4.13
40	54.7	20.6	19.9	9.2	39.8	23.11	121.8	64.2	0.75	4.40
41	58.3	19.5	19.0	10.6	41.9	25.29	124.1	62.8	0.79	3.96
42	61.8	18.9	18.4	9.8	45.9	21.35	133.7	72.5	0.93	4.40
43	87.7	21.0	20.3	9.4	44.6	21.07	137.3	80.4	0.96	4.90
44	100.7	21.6	21.0	9.6	45.8	20.96	143.0	76.8	0.96	4.22
45	88.4	21.0	20.7	9.6	49.9	19.23	135.9	74.9	0.98	4.47
46	66.0	20.7	20.4	7.8	45.7	17.06	148.8	73.1	0.98	4.90
47	59.0	19.3	18.7	9.3	46.7	19.91	127.2	75.9	0.88	4.73
48	108.9	23.1	22.7	9.0	45.4	19.82	132.9	77.8	1.0	4.89
$\bar{X}$	75.0	20.6	20.1	9.8	45.9	21.40	133.7	72.5	0.93	4.40
Sn-1	18.6478	1.2616	1.2769	1.6332	5.4423	2.2577	7.5136	5.2127	0.0872	0.4042
S^2	347.740	1.5917	1.6306	2.6675	29.6190	5.0973	56.4545	27.1729	0.0076	0.1633
$S\bar{x}$	5.3831	0.3642	0.3686	0.4714	1.5710	0.6517	2.1689	1.5047	0.0251	0.1166
U	86.8	21.4	20.9	10.8	49.3	22.83	138.4	75.8	0.99	4.65
L	63.1	19.8	19.3	8.7	42.4	19.96	128.9	69.2	0.88	4.14

Appendix 5: Weight (Wt.,g), total length (T.L, cm), fork length (FL,cm), blood hemoglobin content (Hb,g.dl^{-1}), hematocrit (Hct,%), mean corpuscular hemoglobin concentration (MCHC,g.dl^{-1}), plasma Cl^- and Mg^{+2} (pCl^- and pMg^{+2}, mmol.lit^{-1}) and red cell Cl^- and Mg^{+2} level (rbc Cl and rbc Mg^{+2}, mmol.lit^{-1}) at 5°C, Normoxia, 16L:8D

Fish	Wt.	T.L	F.L	Hb	Hct	MCHC	pCl^-	rbc Cl^-	pMg^{+2}	rbc Mg^{+2}
49	77.8	20.0	19.4	6.4	32.7	19.57	127.9	57.5	0.73	6.79
50	87.7	21.0	20.5	7.0	33.9	20.64	133.2	50.0	0.73	7.05
51	66.6	18.8	18.4	7.3	32.2	22.67	124.0	51.6	0.94	6.45
52	57.1	18.1	17.6	7.0	30.4	23.02	128.9	54.8	0.71	6.62
53	69.8	18.9	18.4	6.7	32.8	20.42	129.6	58.2	0.73	6.96
54	61.0	18.6	18.1	4.6	22.7	20.26	116.2	52.5	0.69	6.11
55	56.8	17.9	17.5	8.4	43.6	19.26	124.7	50.2	0.84	7.04
56	58.6	18.5	18.0	6.5	31.8	20.44	116.8	51.8	0.82	6.36
57	63.6	18.2	17.8	5.5	25.8	21.31	133.5	52.0	0.82	7.47
58	69.3	19.3	18.9	8.4	36.7	22.88	126.9	52.8	0.82	7.05
59	81.7	20.8	20.2	8.0	42.2	18.95	137.8	64.0	0.90	6.79
60	62.3	18.3	17.3	7.7	31.6	24.36	117.8	61.8	0.99	5.67
$\bar{X}$	67.7	19.0	18.5	6.9	33.0	21.14	126.4	54.7	0.81	6.69
Sn-1	10.050	1.0412	1.0457	1.1357	5.8802	1.7090	6.9044	4.6028	0.0963	0.4890
S^2	101.0025	1.0842	1.0935	1.2899	34.5769	2.9206	47.6717	21.1860	9.29×10^{-3}	0.2391
$S_{\bar{x}}$	2.9011	0.3005	0.3018	0.3278	1.6974	0.4933	1.9931	1.3287	0.0278	0.1411
U	74.1	19.7	19.2	7.6	36.7	22.23	130.8	57.7	0.87	7.0
L	61.3	18.4	17.8	6.2	29.3	20.06	122.0	51.8	0.74	6.38

Appendix 6: Weight (Wt.,g), total length (T.L, cm), fork length (FL,cm), blood hemoglobin content ($g.dl^{-1}$), hematocrit (Hct,%), mean corpuscular hemoglobin concentration (MCHC,$g.dl^{-1}$), plasma Cl^- and Mg^{+2} (pCl^- and pMg^{+2}, $mmol.lit^{-1}$) and red cell Cl^- and Mg^{+2} level (rbc Cl^- and rbc Mg^{+2}, $mmol.lit^{-1}$) at 5°C, Normoxia, 8L:16D

Fish	Wt.	T.L	F.L	Hb	Hct	MCHC	pCl^-	rbc Cl^-	pMg^{+2}	rbc Mg^{+2}
61	102.2	21.4	21.0	5.8	19.9	29.14	114.2	59.3	0.61	6.62
62	101.5	22.0	21.8	4.0	16.2	24.69	113.0	51.5	0.65	6.36
63	68.5	19.7	19.0	4.2	19.0	22.10	129.0	72.5	0.73	6.36
64	70.6	18.5	17.8	8.2	36.2	22.65	110.5	60.1	0.64	6.19
65	65.6	18.9	18.1	6.2	26.7	23.22	126.1	56.8	0.76	6.19
66	85.5	20.2	19.7	8.2	38.0	21.57	128.6	62.0	0.79	6.19
67	87.9	20.0	19.7	5.8	27.2	21.32	129.9	50.4	0.77	6.95
68	105.6	22.2	21.8	9.2	42.0	21.90	121.4	59.4	0.52	4.41
69	75.7	19.1	18.5	6.3	30.0	21.0	123.6	64.2	0.78	5.76
70	105.2	22.6	22.1	7.2	31.9	22.57	121.8	57.4	0.78	6.19
71	63.4	19.3	18.7	4.6	23.1	19.91	126.6	68.8	0.71	6.11
72	79.7	19.8	19.0	5.9	27.0	21.85	119.0	56.2	0.92	6.35
$\bar{X}$	84.2	20.3	19.8	6.3	28.1	22.66	121.9	59.8	0.72	6.14
Sn-1	16.0683	1.3931	1.5316	1.6431	7.9516	2.3603	6.5987	6.4093	0.1043	0.6161
S^2	258.1902	1.9408	2.3460	2.70	63.2290	5.5711	43.5438	41.0796	0.0108	0.3795
$\bar{Sx}$	4.6385	0.4021	0.4421	0.4743	2.2954	0.6813	1.9048	1.8502	0.0301	0.1778
U	94.4	21.2	20.7	7.3	33.1	24.15	126.1	63.9	0.78	6.53
L	74.0	19.4	18.8	5.2	23.0	21.16	117.7	55.8	0.65	5.74

Appendix 7: Weight (Wt.,g), total length (T.L, cm), fork length (FL,cm), blood hemoglobin content (Hb,g.dl^{-1}), hematocrit (Hct,%), mean corpuscular hemoglobin concentration (MCHC,g.dl^{-1}), plasma Cl^- and Mg^{+2} (pCl^- and pMg^{+2}, mmol.lit^{-1}) and red cell Cl^- and Mg^{+2} level (rbc Cl^- and rbc Mg^{+2}, mmol.lit^{-1}) at 5°C, Hypoxia, 16L:8D

Fish	Wt.	T.L.	F.L.	Hb	Hct	MCHC	pCl^-	rbc Cl^-	pMg^{+2}	rbc Mg^{+2}
73	66.9	18.5	18.0	7.0	38.4	18.23	120.4	60.4	1.85	5.90
74	63.3	18.8	18.3	4.3	17.6	24.43	108.0	64.9	0.73	5.92
75	64.1	19.0	18.3	5.5	21.2	25.94	123.0	56.3	0.71	5.94
76	68.0	19.6	19.2	9.0	26.5	33.96	116.5	49.8	0.65	6.20
77	45.4	16.8	16.4	8.3	37.3	22.25	92.3	57.9	0.94	5.08
78	58.9	18.6	18.0	5.1	24.4	20.90	129.3	58.8	0.69	5.76
79	55.8	17.9	17.4	7.2	34.2	21.05	125.0	54.9	0.80	6.19
80	74.0	19.7	19.1	5.8	36.9	15.71	114.5	55.9	0.80	5.93
81	39.5	15.9	15.4	6.6	30.5	21.63	110.9	54.0	0.73	5.94
82	92.8	20.8	20.4	9.3	44.5	20.89	123.4	70.4	0.82	5.51
83	72.6	20.0	19.5	7.8	46.0	16.95	92.6	61.9	0.78	5.08
84	49.9	16.8	16.3	10.8	32.3	33.43	85.4	60.1	0.84	5.65
$\bar{X}$	62.6	18.5	18.0	7.2	32.4	22.94	111.7	58.7	0.86	5.75
Sn-1	14.2798	1.4593	1.4585	1.9126	8.8507	5.7774	14.4754	5.3964	0.3207	0.3713
S^2	203.9126	2.1296	2.1274	3.6584	78.3360	33.3786	209.5384	29.122	0.1028	0.1378
$S\bar{x}$	4.1222	0.4212	0.4210	0.5521	2.5549	1.6677	4.1786	1.5578	0.0925	0.1071
U	71.7	19.5	19.0	8.4	38.1	26.6	120.9	62.2	1.06	5.99
L	53.5	17.6	17.1	6.0	26.8	19.27	102.5	55.3	0.65	5.52

Appendix 8: Weight (Wt.,g), total length (T.L, cm), fork length (FL,cm), blood hemoglobin content (Hb,g.dl^{-1}), hematocrit (Hct,%), mean corpuscular hemoglobin concentration (MCHC, g.dl^{-1}), plasma Cl^- and Mg^{+2} (pCl^- and pMg^{+2}, mmol.lit^{-1}) and red cell Cl^- and Mg^{+2} level (rbc Cl^- and rbc Mg^{+2}, mmol.lit^{-1}) at 5°C, Hypoxia, 8L:16D

Fish	Wt.	T.L.	F.L.	Hb	Hct	MCHC	pCl^-	rbc Cl^-	pMg^{+2}	rbc Mg^{+2}
85	69.4	19.0	18.4	8.3	39.6	20.95	130.9	75.6	0.86	5.24
86	63.9	18.9	17.9	6.5	30.1	21.59	122.3	64.2	0.86	4.48
87	54.9	17.7	17.1	8.3	36.5	22.73	129.9	70.2	0.88	4.64
88	60.2	17.9	17.4	8.8	39.9	22.05	123.6	62.8	0.84	4.73
89	85.4	20.0	19.5	9.8	48.5	20.2	130.9	71.5	0.84	5.33
90	98.4	20.5	20.1	8.3	39.8	20.85	137.2	67.9	0.75	5.08
91	95.0	21.2	20.7	11.3	54.2	20.84	94.5	45.3	0.58	5.08
92	73.1	20.6	20.0	8.3	39.7	20.90	129.6	69.3	0.67	4.32
93	75.5	20.8	20.1	9.0	47.0	19.14	123.5	63.5	0.89	4.57
94	68.4	19.8	19.1	7.8	31.3	24.92	117.3	57.8	0.73	5.12
95	75.8	21.3	20.6	8.7	40.2	21.64	120.2	61.6	0.75	4.93
96	101.2	22.7	21.8	9.6	47.6	20.16	129.7	70.5	0.82	5.33
$\bar{X}$	76.7	20.0	19.4	8.7	41.2	21.33	124.1	65.0	0.79	4.90
Sn-1	15.1669	1.4649	1.4418	1.1740	7.0688	1.4700	10.8744	7.9659	0.0949	0.3460
S^2	230.0348	2.1460	2.0790	1.3784	49.9690	2.1610	118.2533	63.456	0.0090	0.1197
$S\bar{x}$	4.3783	0.4228	0.4162	0.3389	2.0406	0.4243	3.1391	2.2995	0.0273	0.0999
U	86.3	20.9	20.3	9.4	45.6	22.26	131.0	70.0	0.85	5.12
L	67.1	19.1	18.5	7.9	36.7	20.39	117.2	59.9	0.72	4.68

Appendix 9: The relationship of erythrocytic electrolytes to hemoglobin ($m$mol. $m$mol Hb^{-1})

20C, normoxia, 16L			20C, normoxia, 8L			20C, hypoxia, 16L			20C, hypoxia, 8L		
No.	Cl^-	Mg^{+2}	No.	Cl^-	Mg^{+2}	No.	Cl^-	Mg^{+2}	No.	Cl^-	Mg^{+2}
1	28.0	0.97	13	27.3	1.40	25	23.7	1.55	37	20.3	1.19
2	22.3	1.27	14	30.3	1.26	26	23.7	1.45	38	20.5	1.06
3	26.9	1.58	15	25.5	1.25	27	24.6	1.04	39	19.2	1.11
4	22.0	1.15	16	30.3	0.96	28	22.5	1.10	40	18.0	1.23
5	27.9	1.40	17	12.3	1.05	29	22.6	1.47	41	16.1	1.01
6	31.9	1.48	18	22.5	1.42	30	23.0	1.14	42	22.0	1.33
7	21.8	1.28	19	19.4	1.48	31	25.6	1.48	43	24.7	1.51
8	26.0	1.42	20	16.6	1.19	32	22.0	1.38	44	23.8	1.30
9	25.4	1.45	21	19.8	1.46	33	28.2	1.26	45	25.3	1.51
10	22.1	1.20	22	16.3	1.44	34	24.9	1.19	46	27.8	1.86
11	28.3	1.26	23	18.5	1.52	35	23.5	1.41	47	24.7	1.54
12	30.0	1.05	24	22.8	1.42	36	24.7	1.50	48	25.5	1.60
$\bar{X}$	26.0	1.29	$\bar{X}$	21.8	1.32	$\bar{X}$	24.0	1.33	$\bar{X}$	22.3	1.35
Sn-1	3.4030	0.1818	Sn-1	5.7058	0.1793	Sn-1	1.6899	0.1758	Sn-1	3.5306	0.2540
S^2	11.5809	0.033	S^2	32.5563	0.0321	S^2	2.8560	0.0309	S^2	12.4656	0.0645
$S\bar{x}$	1.0260	0.0548	$S\bar{x}$	1.7203	0.0540	$S\bar{x}$	0.5095	0.0530	$S\bar{x}$	1.0645	0.0765
U	28.2	1.41	U	25.5	1.43	U	25.1	1.44	U	24.6	1.51
L	23.7	1.16	L	18.0	1.20	L	22.8	1.21	L	19.9	1.18

Appendix 9: (Cont'd..)

$\dot{5}C$, normoxia, 16L			$\dot{5}C$, normoxia, 8L			$\dot{5}C$, hypoxia, 16L			$\dot{5}C$, hypoxia, 8L		
No.	Cl^-	Mg^{+2}	No.	Cl^-	Mg^{+2}	No.	Cl^-	Mg^{+2}	No.	Cl^-	Mg^{+2}
49	19.1	2.25	61	13.2	1.47	73	21.5	2.10	85	23.4	1.62
50	15.7	2.21	62	13.5	1.67	74	17.2	1.57	86	19.3	1.34
51	14.7	1.84	63	21.3	1.87	75	14.0	1.48	87	20.0	1.32
52	15.4	1.86	64	17.2	1.77	76	9.5	1.18	88	18.5	1.39
53	18.5	2.21	65	15.8	1.73	77	16.9	1.48	89	23.0	1.71
54	16.8	1.95	66	18.6	1.86	78	18.2	1.79	90	21.1	1.58
55	16.9	2.37	67	15.3	2.11	79	16.9	1.91	91	14.1	1.58
56	16.4	2.02	68	17.6	1.30	80	23.1	2.45	92	21.5	1.34
57	15.8	2.27	69	19.8	1.78	81	16.2	1.78	93	21.5	1.55
58	14.9	2.0	70	16.5	1.78	82	21.8	1.71	94	15.0	1.33
59	21.9	2.32	71	22.4	1.99	83	23.7	1.94	95	18.5	1.48
60	16.4	1.51	72	16.7	1.88	84	11.6	1.09	96	22.7	1.71
$\bar{X}$	16.8	2.06	$\bar{X}$	17.3	1.76	$\bar{X}$	17.5	1.70	$\bar{X}$	19.8	1.49
Sn-1	2.0591	0.2516	Sn-1	2.8371	0.2167	Sn-1	4.4549	0.3807	Sn-1	2.9878	0.1485
S^2	4.2402	0.0633	S^2	8.0493	0.0469	S^2	19.8463	0.1449	S^2	8.9269	0.0220
$S\bar{x}$	0.6208	0.0758	$S\bar{x}$	0.8554	0.0653	$S\bar{x}$	1.3432	0.1147	$S\bar{x}$	0.9008	0.0447
U	18.1	2.22	U	19.1	1.90	U	20.4	1.95	U	21.7	1.58
L	15.4	1.89	L	15.4	1.61	L	14.5	1.44	L	17.8	1.39

Appendix 10: Rx values for rainbow trout acclimated at 20°C, normoxia, 16L:8D.

Fish	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
1	1.218	1.082	1.002	0.924	0.863	0.782	0.711	0.242	0.135	0.286	0.466	0.606
2	1.206	1.086	1.006	0.926	0.856	0.775	0.724	0.204	0.128	0.277	0.457	0.587
3	1.208	0.079	0.998	0.907	0.847	0.777	0.719	0.219	0.129	0.287	0.477	0.607
4	1.227	1.106	1.018	0.938	0.858	0.787	0.716	0.196	0.142	0.292	0.472	0.602
5	1.227	1.098	1.013	0.923	0.860	0.787	0.702	0.168	0.168	0.305	0.478	0.628
6	1.198	1.076	0.994	0.917	0.847	0.781	0.707	0.187	0.164	0.311	0.469	0.619
7	1.193	1.067	0.995	0.919	0.841	0.779	0.681	0.210	0.138	0.279	0.441	0.591
8	1.230	1.067	0.973	0.914	0.848	0.766	0.717	0.232	0.140	0.288	0.452	0.612
9	1.229	1.069	0.990	0.938	0.877	0.785	0.714	0.259	0.124	0.297	0.488	0.568
10	1.197	1.077	0.998	0.909	0.845	0.775	0.695	0.185	0.167	0.312	0.462	0.622
11	1.233	1.092	1.013	0.909	0.879	0.788	0.730	0.276	0.098	0.257	0.457	0.617
12	1.212	1.092	1.012	0.924	0.834	0.764	0.695	0.185	0.177	0.317	0.467	0.627
$\bar{X}$	1.215	1.082	1.001	0.920	0.854	0.779	0.709	0.213	0.142	0.292	0.465	0.607
Sn-1	0.015	0.012	0.012	0.010	0.013	0.007	0.013	0.032	0.022	0.017	0.012	0.017
S^2	0.20×10^{-3}	0.15×10^{-3}	0.15×10^{-3}	0.10×10^{-3}	0.18×10^{-3}	0.63×10^{-4}	0.19×10^{-3}	0.10×10^{-2}	0.52×10^{-3}	0.29×10^{-3}	0.16×10^{-3}	3.2×10^{-4}
$\bar{Sx}$	0.004	0.003	0.003	0.003	0.004	0.002	0.004	0.009	0.006	0.005	0.003	0.005
U	1.224	1.090	1.009	0.927	0.863	0.784	0.718	0.235	0.157	0.304	0.474	0.619
L	1.205	1.074	0.993	0.914	0.845	0.774	0.70	0.192	0.127	0.281	0.457	0.595

Appendix 11: Rx values for rainbow trout acclimated at 20 °C, normoxia, 8L:16D.

Fish	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
13	1.195	1.035	0.975	0.895	0.825	0.755	0.695	0.235	0.136	0.296	0.475	0.603
14	1.317	1.105	1.035	0.925	0.855	0.785	0.715	0.235	0.125	0.275	0.465	0.619
15	1.245	1.075	1.005	0.915	0.890	0.810	0.730	0.215	0.162	0.302	0.470	0.627
16	1.205	1.055	0.954	0.904	0.854	0.784	0.694	0.214	0.130	0.290	0.450	0.602
17	1.232	1.062	0.995	0.906	0.830	0.758	0.687	0.218	0.133	0.290	0.446	0.588
18	1.219	1.082	1.002	0.927	0.865	0.794	0.713	0.245	0.132	0.297	0.477	0.593
19	1.250	1.076	1.010	0.943	0.847	0.788	0.722	0.217	0.152	0.315	0.510	0.589
20	1.283	1.121	1.040	0.959	0.872	0.809	0.719	0.231	0.184	0.346	0.560	0.630
21	1.234	1.101	1.022	0.933	0.858	0.799	0.714	0.199	0.174	0.309	0.529	0.589
22	1.180	1.044	0.991	0.898	0.832	0.775	0.713	0.233	0.114	0.259	0.436	0.580
23	1.191	1.049	0.980	0.901	0.823	0.745	0.696	0.20	0.147	0.308	0.480	0.553
24	1.193	1.064	0.980	0.896	0.831	0.757	0.702	0.207	0.163	0.311	0.490	0.559
$\bar{X}$	1.229	1.072	0.999	0.917	0.849	0.780	0.708	0.221	0.146	0.30	0.482	0.594
Sn-1	0.040	0.026	0.025	0.020	0.020	0.021	0.013	0.014	0.021	0.021	0.035	0.023
S^2	0.16×10^{-2}	0.68×10^{-3}	0.64×10^{-3}	0.42×10^{-3}	0.43×10^{-3}	0.47×10^{-3}	0.17×10^{-3}	0.21×10^{-3}	0.45×10^{-3}	0.46×10^{-3}	0.12×10^{-2}	5.5×10^{-4}
$\bar{Sx}$	0.012	0.007	0.007	0.006	0.006	0.006	0.003	0.004	0.006	0.006	0.010	0.007
U	1.256	1.089	1.016	0.930	0.862	0.794	0.717	0.230	0.160	0.314	0.506	0.610
L	1.202	1.055	0.982	0.903	0.835	0.765	0.70	0.211	0.132	0.286	0.459	0.579

Appendix 13: Rx values for rainbow trout acclimated at 20°C, hypoxia, 8L:16D.

Fish	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
37	1.233	1.10	1.013	0.937	0.855	0.783	0.721	0.236	0.168	0.322	0.509	0.617
38	1.229	1.076	0.997	0.902	0.828	0.780	0.696	0.190	0.123	0.281	0.445	0.560
39	1.222	1.086	1.016	0.930	0.855	0.774	0.719	0.201	0.140	0.301	0.525	0.605
40	1.202	1.060	0.990	0.902	0.838	0.768	0.719	0.190	0.124	0.306	0.455	0.585
41	1.192	1.055	0.979	0.883	0.828	0.759	0.691	0.196	0.127	0.289	0.451	0.582
42	1.231	1.073	1.026	0.920	0.853	0.779	0.708	0.219	0.111	0.290	0.492	0.635
43	1.214	1.088	0.975	0.906	0.823	0.765	0.726	0.192	0.154	0.30	0.461	0.595
44	1.20	1.097	1.023	0.923	0.839	0.761	0.717	0.196	0.148	0.296	0.471	0.550
45	1.235	1.106	1.013	0.907	0.879	0.788	0.706	0.210	0.177	0.299	0.434	0.583
46	1.230	1.095	1.025	0.938	0.853	0.787	0.721	0.252	0.101	0.243	0.411	0.569
47	1.206	1.090	1.018	0.923	0.852	0.780	0.715	0.205	0.129	0.287	0.461	0.578
48	1.221	1.078	1.021	0.939	0.863	0.795	0.722	0.267	0.163	0.323	0.510	0.636
$\bar{X}$	1.218	1.083	1.008	0.918	0.847	0.777	0.713	0.213	0.139	0.295	0.469	0.591
Sn-1	0.014	0.015	0.018	0.017	0.016	0.011	0.010	0.025	0.023	0.020	0.034	0.027
S^2	0.21×10^{-3}	0.24×10^{-3}	0.33×10^{-3}	0.3×10^{-3}	0.26×10^{-3}	0.13×10^{-3}	0.11×10^{-3}	0.65×10^{-3}	0.55×10^{-3}	0.43×10^{-3}	0.11×10^{-2}	7.5×10^{-4}
$S\bar{x}$	0.004	0.004	0.005	0.005	0.004	0.003	0.003	0.007	0.007	0.006	0.010	0.008
U	1.228	1.094	1.020	0.929	0.858	0.784	0.720	0.230	0.154	0.308	0.491	0.609
L	1.208	1.073	0.996	0.906	0.836	0.769	0.706	0.196	0.123	0.281	0.446	0.573

Appendix 14: Rx values for rainbow trout acclimated at 5°C, normoxia, 16L:8D.

Fish	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
49	1.235	1.076	1.010	0.916	0.848	0.767	0.704	0.242	0.111	0.257	0.438	0.589
50	1.228	1.076	1.002	0.906	0.835	0.764	0.693	0.184	0.189	0.343	0.532	0.626
51	1.222	1.069	1.003	0.914	0.844	0.774	0.702	0.218	0.142	0.308	0.478	0.591
52	1.209	1.074	0.999	0.916	0.849	0.787	0.711	0.233	0.129	0.267	0.443	0.618
53	1.222	1.072	1.003	0.915	0.843	0.778	0.710	0.237	0.128	0.269	0.456	0.592
54	1.250	1.104	1.026	0.950	0.873	0.792	0.720	0.243	0.132	0.291	0.490	0.602
55	1.209	1.077	0.998	0.909	0.845	0.783	0.721	0.218	0.153	0.305	0.483	0.553
56	1.206	1.069	1.008	0.924	0.853	0.783	0.716	0.254	0.112	0.243	0.440	0.579
57	1.214	1.072	1.005	0.910	0.840	0.768	0.704	0.190	0.186	0.334	0.529	0.621
58	1.201	1.071	1.001	0.914	0.852	0.782	0.704	0.210	0.161	0.295	0.493	0.590
59	1.235	1.077	1.002	0.911	0.843	0.774	0.705	0.242	0.116	0.263	0.450	0.576
60	1.202	1.075	0.992	0.903	0.837	0.770	0.698	0.20	0.154	0.268	0.479	0.590
$\bar{X}$	1.219	1.076	1.004	0.916	0.847	0.777	0.707	0.223	0.143	0.287	0.476	0.594
Sn-1	0.015	0.009	0.008	0.012	0.009	0.008	0.008	0.022	0.026	0.031	0.032	0.020
S^2	0.23×10^{-3}	0.86×10^{-4}	0.68×10^{-4}	0.14×10^{-3}	0.95×10^{-4}	0.75×10^{-4}	0.7×10^{-4}	0.52×10^{-3}	0.70×10^{-3}	0.97×10^{-3}	0.001	4.2×10^{-4}
$\bar{Sx}$	0.004	0.002	0.002	0.003	0.002	0.002	0.002	0.006	0.007	0.009	0.009	0.006
U	1.230	1.082	1.009	0.924	0.853	0.782	0.713	0.238	0.160	0.307	0.497	0.607
L	1.209	1.070	0.999	0.908	0.840	0.771	0.702	0.207	0.125	0.266	0.454	0.580

Appendix 15: Rx values for rainbow trout acclimated at 5°C, normoxia, 8L:16D.

Fish	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
61	1.212	1.072	1.017	0.921	0.852	0.758	0.723	0.253	0.165	0.312	0.491	0.616
62	1.191	1.059	0.978	0.918	0.857	0.790	0.728	0.266	0.151	0.284	0.425	0.554
63	1.184	1.084	0.983	0.921	0.839	0.776	0.719	0.235	0.134	0.294	0.440	0.552
64	1.191	1.072	0.995	0.919	0.862	0.785	0.723	0.201	0.162	0.301	0.502	0.618
65	1.230	1.087	0.993	0.937	0.862	0.772	0.699	0.198	0.137	0.305	0.424	0.592
66	1.241	1.094	1.019	0.907	0.856	0.780	0.701	0.231	0.195	0.287	0.455	0.571
67	1.268	1.100	1.021	0.931	0.832	0.790	0.723	0.248	0.173	0.278	0.453	0.591
68	1.248	1.069	0.995	0.90	0.843	0.781	0.699	0.232	0.181	0.319	0.449	0.591
69	1.205	1.078	0.997	0.921	0.855	0.784	0.703	0.206	0.178	0.285	0.453	0.594
70	1.257	1.093	1.011	0.914	0.842	0.780	0.70	0.195	0.115	0.291	0.490	0.607
71	1.233	1.066	0.992	0.913	0.859	0.768	0.698	0.201	0.10	0.297	0.480	0.596
72	1.232	1.070	0.997	0.911	0.816	0.786	0.707	0.213	0.115	0.304	0.504	0.649
$\bar{X}$	1.224	1.079	1.0	0.918	0.848	0.779	0.710	0.223	0.150	0.296	0.464	0.594
Sn-1	0.027	0.0128	0.014	0.010	0.013	0.009	0.011	0.024	0.030	0.012	0.028	0.027
S^2	0.75×10^{-3}	0.16×10^{-3}	0.19×10^{-3}	0.10×10^{-3}	0.19×10^{-3}	0.89×10^{-4}	0.14×10^{-3}	0.59×10^{-3}	0.91×10^{-3}	0.15×10^{-3}	0.81×10^{-3}	0.0007
$\bar{Sx}$	0.008	0.003	0.004	0.003	0.004	0.002	0.003	0.007	0.009	0.003	0.008	0.0082
U	1.242	1.087	1.009	0.924	0.857	0.785	0.718	0.239	0.170	0.304	0.483	0.612
L	1.206	1.070	0.990	0.911	0.839	0.773	0.702	0.207	0.130	0.288	0.445	0.576

Appendix 16: Rx values for rainbow trout acclimated at 5°C, hypoxia, 16L:8D.

Fish	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
73	1.242	1.084	1.012	0.912	0.841	0.762	0.716	0.206	0.122	0.297	0.443	0.583
74	1.209	1.067	1.004	0.919	0.850	0.784	0.714	0.240	0.149	0.308	0.477	0.626
75	1.192	1.051	0.991	0.913	0.836	0.782	0.714	0.251	0.152	0.30	0.491	0.616
76	1.210	1.071	1.004	0.917	0.851	0.784	0.726	0.209	0.149	0.264	0.461	0.581
77	1.171	1.050	0.974	0.894	0.824	0.772	0.696	0.232	0.157	0.284	0.438	0.581
78	1.203	1.063	0.993	0.911	0.835	0.778	0.707	0.211	0.139	0.297	0.444	0.610
79	1.217	1.082	1.021	0.935	0.850	0.797	0.736	0.220	0.103	0.296	0.435	0.590
80	1.230	1.074	1.0	0.914	0.843	0.774	0.702	0.210	0.162	0.289	0.437	0.593
81	1.189	1.051	0.985	0.901	0.829	0.773	0.699	0.208	0.156	0.294	0.455	0.614
82	1.233	1.081	1.014	0.928	0.854	0.789	0.709	0.250	0.150	0.282	0.465	0.605
83	1.249	1.102	1.040	0.957	0.868	0.787	0.735	0.249	0.137	0.289	0.439	0.582
84	1.236	1.106	1.043	0.958	0.868	0.785	0.723	0.228	0.145	0.271	0.467	0.626
$\bar{X}$	1.215	1.074	1.007	0.921	0.846	0.781	0.715	0.226	0.143	0.289	0.479	0.598
Sn-1	0.023	0.018	0.020	0.019	0.013	0.009	0.013	0.017	0.016	0.012	0.082	0.016
S^2	0.56×10^{-3}	0.34×10^{-3}	0.43×10^{-3}	0.39×10^{-3}	0.19×10^{-3}	0.87×10^{-4}	0.17×10^{-3}	0.31×10^{-3}	0.27×10^{-3}	0.15×10^{-3}	0.006	0.0002
$S\bar{x}$	0.007	0.005	0.006	0.005	0.004	0.002	0.003	0.005	0.005	0.003	0.024	0.005
U	1.230	1.086	1.021	0.934	0.855	0.787	0.723	0.238	0.154	0.297	0.534	0.609
L	1.199	1.061	0.993	0.908	0.837	0.774	0.706	0.214	0.132	0.281	0.424	0.587

Appendix 17: Rx values for rainbow trout acclimated at 5°C, hypoxia, 8L:16D.

Fish	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
85	1.235	1.088	0.994	0.916	0.852	0.760	0.710	0.198	0.127	0.292	0.486	0.629
86	1.224	1.087	0.993	0.937	0.823	0.771	0.697	0.194	0.169	0.283	0.450	0.608
87	1.212	1.073	0.979	0.917	0.846	0.770	0.710	0.217	0.146	0.279	0.467	0.615
88	1.218	1.079	0.980	0.938	0.877	0.762	0.708	0.203	0.112	0.286	0.472	0.628
89	1.221	1.079	1.006	0.901	0.831	0.779	0.696	0.20	0.194	0.287	0.481	0.630
90	1.225	1.083	1.005	0.892	0.876	0.784	0.695	0.20	0.130	0.282	0.412	0.580
91	1.208	1.077	1.016	0.914	0.847	0.781	0.706	0.191	0.149	0.295	0.439	0.585
92	1.226	1.096	1.076	0.905	0.840	0.786	0.736	0.246	0.147	0.309	0.423	0.582
93	1.209	1.069	0.993	0.910	0.871	0.779	0.730	0.237	0.152	0.316	0.490	0.620
94	1.198	1.058	0.980	0.909	0.850	0.778	0.723	0.236	0.146	0.290	0.447	0.594
95	1.208	1.067	0.984	0.920	0.870	0.786	0.739	0.253	0.129	0.283	0.478	0.605
96	1.190	1.080	0.998	0.913	0.854	0.774	0.702	0.237	0.168	0.309	0.468	0.601
$\bar{X}$	1.214	1.078	1.0	0.914	0.853	0.776	0.712	0.218	0.147	0.292	0.480	0.606
Sn-1	0.012	0.010	0.026	0.013	0.017	0.008	0.015	0.022	0.021	0.012	0.022	0.018
S^2	0.16×10^{-3}	0.10×10^{-3}	0.69×10^{-3}	0.17×10^{-3}	0.31×10^{-3}	0.74×10^{-4}	0.24×10^{-3}	0.5×10^{-3}	0.48×10^{-3}	0.14×10^{-3}	0.5×10^{-3}	3.4×10^{-4}
$S\bar{x}$	0.003	0.003	0.007	0.004	0.005	0.002	0.004	0.006	0.006	0.003	0.006	0.005
U	1.223	1.084	1.017	0.923	0.865	0.781	0.733	0.232	0.162	0.30	0.495	0.618
L	1.206	1.071	0.983	0.905	0.841	0.770	0.702	0.203	0.133	0.284	0.465	0.594

Appendix 18: Estimated content of hemoglobin polymorph of rainbow trout acclimated at 20°C, Normoxia, 16L:8D ($g.dl^{-1}$)

Fish	Total	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
1	7.6	0.07	0.38	0.57	0.71	2.27	1.21	0.08	0.09	0.87	0.21	1.07	0.10
2	8.1	0.06	0.43	0.81	0.71	1.96	1.38	0.14	0.04	0.66	0.05	1.18	0.06
3	9.8	0.06	0.33	0.61	0.90	3.04	2.05	0.12	0.04	0.87	0.12	1.63	0.10
4	8.7	0.08	0.34	0.61	0.84	2.77	1.74	0.11	0.06	0.71	0.10	1.32	0.07
5	4.5	0.05	0.31	0.43	0.46	1.26	0.60	0.03	0.05	0.46	0.11	0.74	0.03
6	7.0	0.05	0.34	0.45	0.70	2.01	0.78	0.04	0.12	0.87	0.11	1.44	0.08
7	7.0	0.12	0.66	0.58	0.92	1.76	0.69	0.03	0.04	0.60	0.07	1.50	0.02
8	6.5	0.08	0.55	0.67	0.67	1.63	0.80	0.02	0.02	0.59	0.12	1.41	0.03
9	5.5	0.05	0.34	0.41	0.47	1.34	0.87	0.06	0.06	0.59	0.14	1.0	0.05
10	6.5	0.05	0.33	0.48	0.61	1.82	1.22	0.03	0.05	0.75	0.07	1.1	0.03
11	5.5	0.09	0.45	0.48	0.64	1.36	0.70	0.07	0.07	0.54	0.14	0.93	0.04
12	4.6	0.05	0.28	0.41	0.43	1.29	0.94	0.09	0.02	0.33	0.04	0.64	0.08
$\bar{X}$	6.7	0.06	0.39	0.54	0.67	1.87	1.08	0.06	0.05	0.65	0.10	1.16	0.05
Sn-1	1.6153	0.0217	0.1114	0.1218	0.1636	0.5790	0.4534	0.0401	0.0284	0.1703	0.0463	0.3075	0.0283
S^2	2.6093	4.74×10^{-4}	0.0124	0.0148	0.0267	0.3352	0.2056	1.61×10^{-3}	8.09×10^{-4}	0.0290	2.15×10^{-3}	0.0945	8.02×10^{-4}
$S\bar{x}$	0.4663	6.29×10^{-3}	0.0321	0.0351	0.0472	0.1671	0.1309	0.0116	8.21×10^{-3}	0.0491	0.0133	0.0887	8.17×10^{-3}
U	7.8	0.08	0.46	0.62	0.77	2.24	1.36	0.09	0.07	0.76	0.13	1.35	0.07
L	5.7	0.05	0.32	0.46	0.56	1.50	0.79	0.04	0.03	0.54	0.07	0.96	0.03

Appendix 19: Estimated content of hemoglobin polymorph of rainbow trout acclimated at 20°C, Normoxia, 8L:16D ($g.dl^{-1}$)

Fish	Total	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
13	7.4	0.06	0.42	0.70	0.79	2.24	1.60	0.14	0.06	0.42	0.07	1.06	0.04
14	7.9	0.10	0.57	0.61	0.85	2.0	1.02	0.10	0.07	0.91	0.12	1.53	0.03
15	10.0	0.08	0.56	1.02	1.0	2.79	1.71	0.11	0.05	0.89	0.14	1.75	0.06
16	8.1	0.10	0.58	0.81	0.68	2.14	1.38	0.17	0.10	0.85	0.20	0.91	0.06
17	9.3	0.06	0.75	0.71	1.18	2.60	1.28	0.02	0.01	0.59	0.03	2.20	0.02
18	5.5	0.05	0.39	0.55	0.56	1.42	0.75	0.02	0.05	0.62	0.07	1.12	0.02
19	7.3	0.07	0.55	0.71	0.82	2.04	0.90	0.01	0.06	0.83	0.08	1.34	0.04
20	6.8	0.03	0.35	0.63	0.61	1.73	1.12	0.03	0.09	0.94	0.15	1.18	0.02
21	6.2	0.06	0.46	0.60	0.69	1.60	0.90	0.04	0.03	0.66	0.05	1.12	0.05
22	7.3	0.05	0.54	0.63	0.71	1.97	0.97	0.01	0.05	0.87	0.02	1.65	0.01
23	8.2	0.05	0.54	0.66	0.83	2.32	1.20	0.03	0.11	0.96	0.07	1.56	0.03
24	8.8	0.08	0.59	0.81	0.80	2.39	1.34	0.04	0.03	0.72	0.04	2.0	0.06
$\bar{X}$	7.7	0.06	0.52	0.70	0.79	2.10	1.18	0.06	0.06	0.77	0.08	1.45	0.03
Sn-1	1.2723	0.0210	0.1071	0.1274	0.1695	0.3999	0.2936	0.0551	0.0296	0.1681	0.0546	0.3999	0.0177
S^2	1.6187	4.44×10^{-4}	0.0114	0.0162	0.0287	0.1599	0.0862	3.03×10^{-3}	8.81×10^{-4}	0.0282	2.98×10^{-3}	0.1599	3.15×10^{-4}
$\bar{Sx}$	0.3672	6.08×10^{-3}	0.0309	0.0367	0.0489	0.1154	0.0847	0.0159	8.56×10^{-3}	0.0485	0.0157	0.1154	5.12×10^{-3}
U	8.5	0.07	0.59	0.78	0.90	2.35	1.36	0.09	0.07	0.87	0.12	1.70	0.04
L	6.9	0.05	0.45	0.62	0.68	1.84	0.99	0.02	0.04	0.66	0.05	1.19	0.02

Appendix 20: Estimated content of hemoglobin polymorph of rainbow trout acclimated at 20°C, Hypoxia, 16L:8D ($g.dl^{-}$)

Fish	Total	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
25	7.5	0.07	0.65	0.59	0.95	2.06	0.86	0.01	0.03	0.71	0.04	1.63	0.02
26	6.7	0.01	0.40	0.61	0.67	1.76	1.17	0.01	0.03	0.73	0.01	1.19	0.01
27	7.7	0.06	0.45	0.67	0.68	2.17	1.11	0.03	0.10	0.96	0.03	1.41	0.03
28	8.8	0.06	0.62	0.87	0.80	2.36	1.22	0.03	0.09	1.03	0.03	1.73	0.02
29	6.0	0.03	0.43	0.56	0.78	1.65	0.90	0.03	0.02	0.53	0.10	0.96	0.01
30	6.7	0.04	0.34	0.48	0.63	1.83	1.03	0.05	0.12	0.89	0.08	1.16	0.02
31	8.8	0.04	0.53	0.79	0.72	2.37	1.31	0.04	0.09	1.04	0.06	1.77	0.04
32	9.3	0.11	0.74	0.77	0.98	2.44	1.13	0.05	0.18	1.10	0.29	1.36	0.02
33	6.7	0.04	0.37	0.67	0.49	1.83	1.11	0.05	0.09	0.77	0.06	1.15	0.02
34	6.0	0.02	0.30	0.41	0.74	1.94	0.91	0.04	0.07	0.61	0.06	0.85	0.02
35	7.3	0.06	0.44	0.64	0.79	1.84	1.30	0.12	0.14	0.60	0.15	1.14	0.08
36	7.5	0.06	0.67	0.58	0.80	2.09	0.90	0.02	0.02	0.72	0.06	1.61	0.02
$\bar{X}$	7.4	0.05	0.49	0.63	0.75	2.02	1.07	0.04	0.08	0.80	0.08	1.33	0.02
Sn-1	1.0886	0.0262	0.1438	0.1299	0.1330	0.2625	0.1589	0.0289	0.0506	0.1913	0.0753	0.3035	0.0188
S^2	1.1851	6.90×10^{-4}	0.0206	0.0168	0.0176	0.0689	0.0252	8.36×10^{-4}	2.56×10^{-3}	0.0366	5.68×10^{-3}	0.0921	3.53×10^{-4}
$S\bar{x}$	0.3142	7.58×10^{-3}	0.0415	0.0375	0.0383	0.0757	0.0458	8.34×10^{-3}	0.0146	0.0552	0.0217	0.0876	5.42×10^{-3}
U	8.1	0.06	0.58	0.72	0.83	2.19	1.18	0.05	0.11	0.93	0.12	1.52	0.03
L	6.7	0.03	0.40	0.55	0.66	1.86	0.97	0.02	0.05	0.68	0.03	1.13	0.01

Appendix 21: Estimated content of hemoglobin polymorph of rainbow trout acclimated at 20°C, Hypoxia, 8L:16D ($g.dl^{-1}$)

Fish	Total	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
37	9.5	0.06	0.61	0.77	0.91	2.59	1.27	0.10	0.14	1.05	0.29	1.46	0.11
38	9.5	0.09	0.69	0.88	0.91	2.40	1.22	0.08	0.10	1.01	0.37	1.55	0.08
39	14.6	0.20	1.1	1.2	1.50	4.20	1.40	0.10	0.20	1.5	0.25	2.70	0.10
40	9.2	0.07	0.77	0.83	1.11	2.40	1.14	0.08	0.05	0.85	0.20	1.54	0.04
41	10.6	0.10	0.80	0.92	1.12	2.82	1.32	0.12	0.10	1.10	0.15	1.87	0.10
42	9.8	0.12	0.68	1.05	1.07	2.70	1.40	0.09	0.03	0.83	0.20	1.60	0.06
43	9.4	0.11	0.77	0.84	1.0	2.31	1.19	0.09	0.07	0.89	0.12	1.91	0.09
44	9.6	0.07	0.66	0.95	0.76	2.64	1.43	0.03	0.12	1.19	0.08	1.72	0.03
45	9.6	0.05	0.65	0.81	0.99	2.51	1.40	0.07	0.16	1.17	0.19	1.67	0.05
46	7.8	0.05	0.54	0.67	0.83	2.13	1.20	0.12	0.07	0.77	0.07	1.53	0.05
47	9.3	0.06	0.80	0.83	1.22	2.62	1.31	0.11	0.04	0.82	0.05	1.56	0.07
48	9.0	0.05	0.63	0.83	0.81	2.45	1.23	0.03	0.11	1.27	0.08	1.82	0.05
$\bar{X}$	9.8	0.08	0.72	0.88	1.01	2.64	1.29	0.08	0.09	1.03	0.17	1.74	0.07
Sn-1	1.6332	0.0433	0.1431	0.1374	0.2054	0.5230	0.0984	0.030	0.0508	0.2200	0.0983	0.3343	0.0264
S^2	2.6675	1.88×10^{-3}	0.0204	0.0189	0.0422	0.2735	9.69×10^{-3}	9×10^{-4}	2.59×10^{-3}	0.0484	9.68×10^{-3}	0.1117	6.99×10^{-4}
$S\bar{x}$	0.4714	0.0125	0.0413	0.0396	0.0593	0.1509	0.0284	8.66×10^{-3}	0.0146	0.0635	0.0284	0.0965	7.63×10^{-3}
U	10.8	0.11	0.81	0.96	1.14	2.97	1.35	1.04	0.13	1.17	0.23	1.95	0.08
L	8.7	0.05	0.63	0.79	0.88	2.31	1.22	0.06	0.06	0.89	0.10	1.53	0.05

Appendix 22: Estimated content of hemoglobin polymorph of rainbow trout acclimated at 5°C, Normoxia, 16L:8D ($g.dl^{-1}$)

Fish	Total	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
49	6.4	0.06	0.48	0.61	0.64	1.60	0.92	0.07	0.04	0.76	0.14	1.26	0.03
50	7.0	0.04	0.40	0.69	0.52	1.67	1.25	0.05	0.08	1.06	0.16	1.29	0.04
51	7.3	0.08	0.60	0.65	0.79	1.83	0.89	0.03	0.06	0.82	0.16	1.51	0.02
52	7.0	0.09	0.64	0.62	0.91	1.84	0.78	0.04	0.03	0.53	0.05	1.57	0.03
53	6.7	0.04	0.42	0.64	0.66	1.81	1.14	0.03	0.05	0.78	0.02	1.32	0.04
54	4.6	0.07	0.41	0.44	0.52	1.20	0.50	0.02	0.03	0.45	0.03	1.03	0.03
55	8.4	0.06	0.68	0.75	0.95	2.30	1.18	0.05	0.03	0.80	0.19	1.52	0.06
56	6.5	0.06	0.53	0.58	0.82	1.83	0.77	0.03	0.02	0.58	0.03	1.41	0.03
57	5.5	0.05	0.23	0.34	0.69	1.64	0.77	0.02	0.03	0.61	0.02	1.20	0.02
58	8.4	0.06	0.49	0.81	0.64	2.17	1.33	0.04	0.13	1.10	0.05	1.67	0.05
59	8.0	0.09	0.63	0.72	0.74	1.99	1.09	0.05	0.06	0.99	0.14	1.74	0.06
60	7.7	0.04	0.45	0.78	0.66	1.98	1.29	0.06	0.07	0.99	0.12	1.48	0.07
$\bar{X}$	6.9	0.06	0.49	0.63	0.71	1.82	0.99	0.04	0.05	0.78	0.09	1.41	0.04
Sn-1	1.1357	0.0180	0.1279	0.1362	0.1363	0.2853	0.2589	0.0156	0.0307	0.2154	0.0645	0.2049	0.0165
S^2	1.2899	3.24×10^{-4}	0.0163	0.0185	0.0185	0.0814	0.0670	2.44×10^{-4}	9.47×10^{-4}	0.0464	4.16×10^{-3}	0.0420	2.72×10^{-4}
$\bar{Sx}$	0.3278	5.19×10^{-3}	0.0369	0.0393	0.0393	0.0823	0.0747	4.51×10^{-3}	8.88×10^{-3}	0.0622	0.0186	0.0591	4.76×10^{-3}
U	7.6	0.07	0.57	0.72	0.79	2.0	1.15	0.05	0.07	0.92	0.13	1.54	0.05
L	6.2	0.05	0.41	0.54	0.62	1.64	0.82	0.03	0.03	0.65	0.05	1.28	0.03

Appendix 23: Estimated content of hemoglobin polymorph of rainbow trout acclimated at 5°C, Normoxia, 8L:16D ($g.dl^{-}$)

Fish	Total	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
61	5.8	0.07	0.43	0.54	0.49	1.62	0.70	0.04	0.03	0.51	0.15	1.22	0.04
62	4.0	0.02	0.20	0.41	0.34	1.13	0.60	0.04	0.02	0.38	0.09	0.77	0.07
63	4.2	0.01	0.18	0.25	0.52	1.12	0.71	0.04	0.02	0.45	0.10	0.79	0.06
64	8.2	0.02	0.41	0.50	1.01	2.28	1.01	0.08	0.10	1.0	0.22	1.60	0.09
65	6.2	0.02	0.29	0.40	0.60	1.95	0.90	0.05	0.09	0.75	0.12	1.02	0.03
66	8.2	0.07	0.44	0.84	0.52	2.06	1.37	0.05	0.14	1.09	0.12	1.46	0.02
67	5.8	0.05	0.43	0.57	0.50	1.65	0.72	0.01	0.04	0.63	0.02	1.22	0.04
68	9.2	0.06	0.55	0.79	0.72	2.37	1.25	0.07	0.09	1.15	0.09	1.97	0.06
69	6.3	0.06	0.41	0.62	0.51	1.74	0.94	0.07	0.02	0.60	0.03	1.28	0.06
70	7.2	0.01	0.32	0.45	0.69	2.2	0.93	0.04	0.11	0.99	0.13	1.37	0.02
71	4.6	0.04	0.32	0.43	0.35	1.29	0.60	0.02	0.02	0.43	0.12	0.98	0.02
72	5.9	0.05	0.34	0.55	0.40	1.61	0.74	0.04	0.14	0.84	0.12	1.03	0.03
$\bar{X}$	6.3	0.04	0.36	0.52	0.55	1.75	0.87	0.04	0.06	0.73	0.10	1.22	0.04
Sn-1	1.6431	0.0229	0.1060	0.1653	0.1854	0.4309	0.2455	0.0202	0.0482	0.2738	0.0521	0.3452	0.0227
S^2	2.70	5.27×10^{-4}	0.0112	0.0273	0.0344	0.1857	0.0602	4.08×10^{-4}	2.32×10^{-3}	0.0749	2.71×10^{-3}	0.1192	5.18×10^{-4}
$S\bar{x}$	0.4743	6.62×10^{-3}	0.0306	0.0477	0.0535	0.1244	0.0708	5.83×10^{-3}	0.0139	0.0790	0.0150	0.0996	6.57×10^{-3}
U	7.3	0.05	0.42	0.63	0.67	2.02	1.02	0.05	0.09	0.90	0.14	1.44	0.06
L	5.2	0.02	0.29	0.42	0.43	1.47	0.71	0.03	0.03	0.56	0.07	1.0	0.03

Appendix 24: Estimated content of hemoglobin polymorph of rainbow trout acclimated at 5°C, Hypoxia, 16L:8D ($g.dl^{-1}$)

Fish	Total	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
73	7.0	0.07	0.54	0.44	0.86	1.92	1.06	0.07	0.06	0.42	0.11	1.48	0.06
74	4.3	0.04	0.29	0.36	0.53	1.09	0.78	0.05	0.05	0.23	0.02	0.78	0.06
75	5.5	0.03	0.38	0.48	0.64	1.28	0.98	0.04	0.05	0.39	0.08	1.04	0.04
76	9.0	0.10	0.69	0.78	0.91	2.25	1.19	0.09	0.12	1.11	0.09	1.75	0.15
77	8.3	0.07	0.55	0.78	0.96	2.09	1.30	0.07	0.11	0.62	0.10	1.53	0.05
78	5.1	0.03	0.33	0.50	0.53	1.36	0.80	0.04	0.07	0.38	0.05	1.02	0.05
79	7.2	0.13	0.71	0.66	1.09	1.86	0.80	0.03	0.01	0.40	0.15	1.46	0.08
80	5.8	0.03	0.36	0.48	0.64	1.47	0.88	0.07	0.07	0.56	0.08	1.09	0.10
81	6.6	0.10	0.46	0.62	0.58	1.41	1.01	0.12	0.06	0.77	0.11	1.32	0.06
82	9.3	0.11	0.71	0.81	0.81	2.29	1.35	0.12	0.12	0.98	0.12	1.73	0.08
83	7.8	0.06	0.73	0.69	1.16	2.08	1.0	0.11	0.02	0.38	0.07	1.51	0.09
84	10.8	0.10	0.50	1.0	0.93	2.83	2.03	0.10	0.10	0.80	0.10	2.4	0.20
$\bar{X}$	7.2	0.07	0.52	0.63	0.80	1.82	1.09	0.07	0.07	0.58	0.09	1.42	0.08
Sn-1	1.9126	0.0351	0.1608	0.1882	0.2168	0.5138	0.3490	0.0320	0.0364	0.2736	0.0338	0.4288	0.0468
S^2	3.6584	1.23×10^{-3}	0.0258	0.0354	0.0470	0.2640	0.1218	1.02×10^{-3}	1.32×10^{-3}	0.0748	1.14×10^{-3}	0.1839	2.19×10^{-3}
$S\bar{x}$	0.5521	0.0101	0.0464	0.0543	0.0625	0.1483	0.1007	9.24×10^{-3}	0.0105	0.0789	9.77×10^{-3}	0.1238	0.0135
U	8.4	0.09	0.62	0.75	0.94	2.15	1.32	0.09	0.09	0.76	0.11	1.69	0.11
L	6.0	0.05	0.41	0.51	0.66	1.50	0.87	0.05	0.04	0.41	0.06	1.15	0.05

Appendix 25: Estimated content of hemoglobin polymorph of rainbow trout acclimated at 5°C, Hypoxia, 8L:16D ($g.dl^{-1}$)

Fish	Total	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
85	8.3	0.11	0.71	0.67	0.89	2.23	1.19	0.14	0.03	0.44	0.07	1.66	0.07
86	6.5	0.07	0.45	0.59	0.60	1.63	1.03	0.05	0.03	0.45	0.10	1.05	0.04
87	8.3	0.09	0.55	0.78	0.74	2.23	1.30	0.08	0.11	0.89	0.10	1.41	0.04
88	8.8	0.05	0.66	0.69	0.97	2.28	1.17	0.05	0.10	0.35	0.09	1.80	0.06
89	9.8	0.06	0.75	0.83	1.0	2.96	1.40	0.05	0.11	0.59	0.06	2.12	0.02
90	8.3	0.05	0.42	0.83	0.98	2.43	0.97	0.02	0.12	1.0	0.03	1.47	0.02
91	11.3	0.10	0.50	1.10	0.82	3.0	1.92	0.10	0.10	1.52	0.15	2.0	0.10
92	8.3	0.10	0.69	0.80	0.90	2.20	1.23	0.12	0.10	0.40	0.10	1.56	0.10
93	9.0	0.11	0.71	0.69	1.0	2.30	1.21	0.09	0.09	0.52	0.12	2.0	0.16
94	7.8	0.07	0.64	0.55	0.60	2.43	1.12	0.03	0.10	0.39	0.08	1.73	0.03
95	8.7	0.06	0.70	0.68	0.95	2.30	1.22	0.04	0.10	0.51	0.08	1.98	0.07
96	9.6	0.10	0.68	0.82	1.15	2.51	1.22	0.13	0.11	0.68	0.15	2.20	0.05
$\bar{X}$	8.7	0.08	0.62	0.75	0.88	2.37	1.24	0.07	0.09	0.64	0.09	1.74	0.06
Sn-1	1.1740	0.0231	0.1120	0.1438	0.1665	0.3574	0.2395	0.0407	0.0297	0.3413	0.0347	0.3368	0.0407
S^2	1.3784	5.35×10^{-4}	0.0125	0.0206	0.0277	0.1277	0.0573	1.66×10^{-3}	8.87×10^{-4}	0.1165	1.20×10^{-3}	0.1134	1.66×10^{-3}
$S\bar{x}$	0.3389	6.68×10^{-3}	0.0323	0.0415	0.0480	0.1031	0.0691	0.0117	8.60×10^{-3}	0.0985	0.0100	0.0972	0.0117
U	9.4	0.09	0.69	0.84	0.98	2.60	1.40	0.10	0.11	0.86	0.11	1.96	0.08
L	7.9	0.06	0.55	0.66	0.77	2.14	1.09	0.05	0.07	0.42	0.07	1.53	0.03

Appendix 26: Hemoglobin polymorph percentage content of rainbow trout acclimated at 20 °C, Normoxia, 16L:8D

Fish	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
1	0.92	5.0	7.50	9.34	29.86	15.92	1.05	1.18	11.44	2.76	14.07	1.31
2	0.74	5.30	10.0	8.76	24.19	17.03	1.72	0.49	8.14	0.61	14.56	0.74
3	0.61	3.36	6.22	9.18	31.02	20.91	1.22	0.40	8.87	1.22	16.63	1.02
4	0.92	3.90	7.01	9.65	31.83	20.0	1.26	0.69	8.16	1.15	15.17	0.80
5	1.11	6.88	9.55	10.22	28.0	13.33	0.66	1.11	10.22	2.44	16.44	0.66
6	0.71	4.85	6.42	10.0	28.71	11.14	0.57	1.71	12.42	1.57	20.57	1.14
7	1.71	9.42	8.28	13.14	25.14	9.85	0.42	0.57	8.57	1.0	21.42	0.28
8	1.23	8.46	10.30	10.30	25.07	12.30	0.30	0.30	9.07	1.84	21.69	0.46
9	0.91	6.18	7.45	8.54	24.36	15.81	1.09	1.09	10.72	2.54	18.18	0.91
10	0.77	5.07	7.38	9.38	28.0	18.77	0.46	0.77	11.53	1.07	16.92	0.46
11	1.63	8.18	8.72	11.63	24.72	12.72	1.27	1.27	9.81	2.54	16.91	0.72
12	1.08	6.08	8.91	9.34	28.04	20.43	1.95	0.43	7.17	0.84	13.91	1.74
$\bar{X}$	1.02	6.05	8.14	9.95	27.41	15.68	0.99	0.83	9.67	1.63	17.20	0.85
Sn-1	0.3490	1.8667	1.3633	1.2916	2.6780	3.8178	0.5256	0.4336	1.6141	0.7606	2.7479	0.4064
S^2	0.1218	3.4847	1.8588	1.6683	7.1717	14.5756	0.2762	0.1880	2.6054	0.5785	7.5510	0.1651
$S\bar{x}$	0.1007	0.5388	0.3935	0.3728	0.7730	1.1021	0.1517	0.1251	0.4659	0.2195	0.7932	0.1173
U	1.25	7.24	9.01	10.77	29.11	18.10	1.33	1.10	10.70	2.11	18.95	1.11
L	0.80	4.87	7.27	9.13	25.71	13.25	0.66	0.55	8.65	1.15	15.45	0.59

Appendix 27: Hemoglobin polymorph percentage content of rainbow trout acclimated at 20°C, Normoxia, 8L:16D

Fish	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
13	0.81	5.67	9.46	10.67	30.27	21.62	1.89	0.81	11.75	2.83	14.46	1.35
14	1.26	7.21	7.72	10.76	25.31	12.91	1.26	0.88	11.51	1.52	19.36	0.38
15	0.80	5.60	10.2	10.0	27.90	17.10	1.10	0.50	8.90	1.40	17.5	0.60
16	1.23	7.16	10.0	8.39	26.42	17.03	2.09	1.23	10.49	2.47	11.23	0.74
17	0.64	8.06	7.63	12.69	27.95	13.76	0.21	0.11	6.34	0.32	23.65	0.21
18	0.91	7.09	10.0	10.18	25.81	13.63	0.36	0.91	11.27	1.27	20.36	0.36
19	0.95	7.53	9.72	11.23	27.94	12.32	0.13	0.82	11.37	1.09	18.35	0.54
20	0.44	5.14	9.26	8.97	25.44	16.47	0.44	1.32	13.82	2.20	17.35	0.29
21	0.96	7.42	9.67	11.13	25.80	14.51	0.64	0.48	10.64	0.80	18.06	0.80
22	0.68	7.39	8.63	9.72	26.98	13.28	0.13	0.68	11.91	0.27	22.60	0.13
23	0.61	6.58	8.04	10.12	28.29	14.63	0.36	1.34	11.70	0.85	19.02	0.36
24	0.91	6.70	9.20	9.09	27.16	15.22	0.45	0.34	8.18	0.45	22.72	0.68
$\bar{X}$	0.85	6.79	9.12	10.24	27.10	15.20	0.75	0.78	10.65	1.28	18.72	0.53
Sn-1	0.2420	0.8926	0.9098	1.1651	1.4562	2.5646	0.6769	0.3882	1.9853	0.8430	3.5256	0.3325
S^2	0.0585	0.7968	0.8277	1.3575	2.1206	6.5774	0.4582	0.1507	3.9414	0.7107	12.4303	0.1106
$S\bar{x}$	0.0698	0.2576	0.2626	0.3363	0.4203	0.7403	0.1954	0.1120	0.5731	0.2433	1.0177	0.0960
U	1.00	7.36	9.70	10.98	28.03	16.83	1.18	1.03	11.91	1.82	20.96	0.74
L	0.69	6.22	8.54	9.50	26.18	13.57	0.32	0.53	9.39	0.75	16.48	0.32

Appendix 28: Hemoglobin polymorph percentage content of rainbow trout acclimated at 20°C, Hypoxia, 16L:8D

Fish	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
25	0.93	8.66	7.86	12.66	27.46	11.46	0.13	0.40	9.46	0.53	21.73	0.26
26	0.15	5.97	9.10	10.0	26.26	17.46	0.15	0.44	10.89	0.15	17.76	0.15
27	0.78	5.84	8.70	8.83	28.18	14.41	0.39	1.29	12.46	0.39	18.31	0.39
28	0.68	7.04	9.88	9.09	26.82	13.86	0.34	1.02	11.36	0.34	19.66	0.22
29	0.50	7.16	9.33	13.0	27.50	15.0	0.50	0.33	8.83	1.66	16.0	0.16
30	0.59	5.07	7.16	9.40	27.31	15.37	0.74	1.79	13.28	1.19	17.31	0.29
31	0.45	6.02	8.97	8.18	26.93	14.88	0.45	1.02	11.81	0.68	20.11	0.45
32	1.18	7.95	8.28	10.53	26.23	12.15	0.53	1.93	11.82	3.11	14.62	0.21
33	0.59	5.52	10.0	7.31	27.31	16.56	0.74	1.34	11.49	0.89	17.16	0.29
34	0.33	5.0	6.83	12.33	32.33	15.16	0.66	1.16	10.16	1.0	14.16	0.33
35	0.82	6.02	8.76	10.82	25.20	17.80	1.64	1.91	8.22	2.05	15.61	1.09
36	0.80	8.93	7.73	10.66	27.86	12.0	0.26	0.26	9.6	0.80	21.46	0.26
$\bar{X}$	0.65	6.59	8.55	10.23	27.44	14.67	0.54	1.07	10.78	1.06	17.82	0.34
Sn-1	0.2780	1.3388	1.0062	1.7924	1.7370	2.0569	0.4017	0.6140	1.5357	0.8463	2.5215	0.2513
S^2	0.0773	1.7924	1.0124	3.2128	3.0174	4.2311	0.1613	0.3770	2.3584	0.7163	6.3580	0.0631
$S_{\bar{x}}$	0.0802	0.3864	0.2904	0.5174	0.5014	0.5937	0.1159	0.1772	0.4433	0.2443	0.7279	0.0725
U	0.82	7.44	9.18	11.37	28.55	15.98	0.80	1.46	11.75	1.60	19.42	0.50
L	0.47	5.74	7.91	9.09	26.34	13.36	0.28	0.68	9.80	0.52	16.22	0.18

Appendix 29: Hemoglobin polymorph percentage content of rainbow trout acclimated at 20°C, Hypoxia, 8L:16D

Fish	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
37	0.63	6.42	8.10	9.57	27.26	13.36	1.05	1.47	11.05	3.05	15.36	1.15
38	0.94	7.26	9.26	9.57	25.26	12.84	0.84	1.05	10.63	3.89	16.31	0.84
39	1.37	7.53	8.22	10.27	28.76	9.59	0.68	1.37	10.27	1.71	18.49	0.68
40	0.76	8.37	9.02	12.06	26.08	12.39	0.87	0.54	9.24	2.17	16.74	0.43
41	0.94	7.54	8.68	10.56	26.60	12.45	1.13	0.94	10.37	1.41	17.64	0.94
42	1.22	6.93	10.71	10.91	27.55	14.28	0.91	0.30	8.47	2.04	16.32	0.61
43	1.17	8.19	8.93	10.63	24.57	12.66	0.95	0.74	9.46	1.27	20.32	0.95
44	0.73	6.87	9.89	7.91	27.50	14.89	0.31	1.25	12.39	0.83	17.91	0.31
45	0.52	6.77	8.43	10.31	26.14	14.58	0.73	1.66	12.18	1.98	17.39	0.52
46	0.64	6.92	8.59	10.64	27.30	15.38	1.55	0.89	9.87	0.89	19.61	0.64
47	0.64	8.60	8.92	13.11	28.17	14.08	1.18	0.43	8.81	0.53	16.77	0.75
48	0.55	7.0	9.22	9.0	27.22	13.66	0.33	1.22	14.11	0.89	20.22	0.55
$\bar{X}$	0.84	7.36	8.99	10.37	26.86	13.34	0.87	0.98	10.57	1.72	17.75	0.69
Sn-1	0.0802	0.6939	0.7297	1.3501	1.1944	1.5405	0.3439	0.4279	0.6458	0.9878	1.6150	0.2405
S^2	0.2833	0.4815	0.5325	1.8230	1.4266	2.3731	0.1182	0.1831	2.7089	0.9759	2.6082	0.0578
$S\bar{x}$	0.0817	0.2003	0.2106	0.3897	0.3447	0.4447	0.0992	0.1235	0.4751	0.2851	0.4662	0.0694
U	1.02	7.80	9.46	11.23	27.62	14.32	1.09	1.26	11.61	2.34	18.78	0.85
L	0.66	6.92	8.53	9.52	26.10	12.36	0.65	0.71	9.52	1.09	16.73	0.54

Appendix 30: Hemoglobin polymorph percentage content of rainbow trout acclimated at 5°C, Normoxia, 16L:8D

Fish	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
49	0.93	7.50	9.53	10.0	25.0	14.37	1.09	0.62	11.87	2.18	19.68	0.46
50	0.57	5.71	9.85	7.42	23.85	17.85	0.71	1.14	15.14	2.28	18.42	0.57
51	1.09	8.22	8.90	10.82	25.06	12.19	0.41	0.82	11.23	2.19	20.68	0.27
52	1.28	9.14	8.85	13.0	26.28	11.14	0.57	0.42	7.57	0.71	22.42	0.42
53	0.59	6.26	9.55	9.85	27.0	17.01	0.44	0.74	11.64	0.29	19.70	0.59
54	1.52	8.91	9.56	11.30	26.08	10.86	0.43	0.65	9.78	0.65	22.39	0.65
55	0.71	8.09	8.92	11.31	27.38	14.04	0.59	0.35	9.52	2.26	18.09	0.71
56	0.92	8.15	8.92	12.61	28.15	11.84	0.46	0.30	8.92	0.46	21.69	0.46
57	0.91	4.18	6.18	12.54	29.81	14.0	0.36	0.54	11.09	0.36	21.81	0.36
58	0.71	5.83	9.64	7.62	25.83	15.83	0.47	1.54	13.09	0.59	19.88	0.59
59	1.12	7.87	9.0	9.25	24.87	13.62	0.62	0.75	12.37	1.75	21.75	0.75
60	0.52	5.84	10.13	8.57	25.71	16.75	0.78	0.91	12.85	1.55	19.22	0.91
$\bar{X}$	0.90	7.14	9.08	10.35	26.25	14.12	0.57	0.73	11.25	1.27	20.47	0.56
Sn-1	0.3068	1.5336	1.0097	1.9011	1.6310	2.3523	0.2055	0.3496	2.0674	0.8305	1.5210	0.1795
S^2	0.0941	2.3519	1.0195	3.6144	2.6603	5.5336	0.0422	0.1222	4.2744	0.6898	2.3135	0.0322
$S\bar{x}$	0.0885	0.4427	0.2914	0.5488	0.4708	0.6790	0.0593	0.1009	0.5968	0.2397	0.4390	0.0518
U	1.10	8.11	9.72	11.56	27.28	15.62	0.70	0.95	12.56	1.80	21.44	0.67
L	0.71	6.16	8.44	9.15	25.21	12.63	0.44	0.51	9.94	0.74	19.51	0.44

Appendix 31: Hemoglobin polymorph percentage content of rainbow trout acclimated at 5 °C, Normoxia, 8L:16D

Fish	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
61	1.20	7.41	9.31	8.44	27.93	12.06	0.69	0.51	8.62	2.58	21.03	0.69
62	0.50	5.0	10.25	8.50	28.25	15.0	1.0	0.50	9.50	2.25	19.25	1.75
63	0.24	4.28	5.95	12.38	26.66	16.90	0.95	0.47	10.71	2.38	18.81	1.42
64	0.24	5.0	6.09	12.31	27.80	12.31	0.97	1.22	12.19	2.68	19.51	1.09
65	0.32	4.67	6.45	9.67	31.45	14.51	0.80	1.45	12.09	1.93	16.45	0.48
66	0.85	5.36	10.24	6.34	25.12	16.70	0.61	1.70	13.29	1.46	17.80	0.24
67	0.86	7.41	9.82	8.62	28.44	12.41	0.17	0.69	10.86	0.34	21.03	0.69
68	0.65	5.97	8.58	7.82	25.76	13.58	0.76	0.97	12.50	0.97	21.41	0.65
69	0.95	6.50	9.84	8.09	27.62	14.92	1.11	0.31	9.52	0.47	20.31	0.95
70	0.14	4.44	6.25	9.58	30.55	12.91	0.55	1.52	13.75	1.80	19.02	0.27
71	0.87	6.95	9.34	7.60	28.04	13.04	0.43	0.43	9.34	2.60	21.30	0.43
72	0.84	5.76	9.32	6.78	27.28	12.54	0.67	2.37	14.23	2.03	17.45	0.50
$\bar{X}$	0.63	5.72	8.45	8.84	27.90	13.90	0.72	1.01	11.38	1.79	19.44	0.76
Sn-1	0.3420	1.1232	1.7377	1.9013	1.7678	1.6859	0.2670	0.6435	1.8900	0.8166	1.6336	0.4619
S^2	0.1170	1.2615	3.0198	3.6151	3.1251	2.8424	0.0713	0.4141	3.5722	0.6668	2.6688	0.2134
$\bar{Sx}$	0.0987	0.3242	0.5016	0.5488	0.5103	0.4866	0.0770	0.1857	0.5456	0.2357	0.4715	0.1333
U	0.85	6.44	9.55	10.05	29.03	14.97	0.89	1.42	12.58	2.31	20.48	1.05
L	0.42	5.01	7.34	7.63	26.78	12.83	0.55	0.60	10.18	1.27	18.41	0.47

Appendix 32: Hemoglobin polymorph percentage content of rainbow trout acclimated at 5°C, Hypoxia, 16L:8D

Fish	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
73	1.0	7.71	6.28	12.28	27.42	15.14	1.0	0.89	6.0	1.57	21.14	0.91
74	0.93	6.74	8.37	12.32	25.35	18.14	1.16	1.16	5.35	0.46	18.14	1.40
75	0.55	6.91	8.72	11.63	23.27	17.81	0.72	0.91	7.09	1.45	18.91	0.72
76	1.11	7.66	8.66	10.11	25.0	13.22	1.0	1.33	12.33	1.0	19.44	1.66
77	0.84	6.62	9.39	11.56	25.18	15.66	0.84	1.32	7.46	1.20	18.40	0.60
78	0.59	6.47	9.80	10.39	26.6	15.68	0.78	1.37	7.45	0.98	20.0	0.98
79	1.80	9.83	9.16	15.13	25.83	11.11	0.41	0.13	5.55	2.08	20.27	1.11
80	0.51	6.20	8.27	11.03	25.34	15.17	1.20	1.20	9.65	1.38	18.79	1.72
81	1.51	6.97	9.39	8.78	21.36	15.30	1.81	0.91	11.66	1.66	20.0	0.91
82	1.18	7.63	8.71	8.71	24.62	14.51	1.29	1.29	10.53	1.29	18.60	0.86
83	0.77	9.35	8.84	14.87	26.66	12.82	1.41	0.25	4.87	0.89	19.35	1.15
84	0.92	4.63	9.26	8.61	26.20	18.79	0.92	0.92	7.40	0.92	22.22	1.85
$\bar{X}$	0.97	7.22	8.73	11.28	25.23	15.27	1.04	0.97	7.94	1.24	19.60	1.15
Sn-1	0.3858	1.3820	0.8965	2.1784	1.6318	2.2464	0.3638	0.4093	2.5196	0.4282	1.1983	0.4105
s^2	0.1489	1.910	0.8038	4.7454	2.6628	5.0467	0.1324	0.1675	6.3483	0.1833	1.4360	0.1685
$S\bar{x}$	0.1113	0.3989	0.2587	0.6288	0.4710	0.6484	0.1050	0.1181	0.7273	0.1236	0.3459	0.1185
U	1.22	8.09	9.29	12.66	26.26	16.69	1.27	1.23	9.54	1.51	20.36	1.41
L	0.73	6.34	8.16	9.89	24.19	13.84	0.80	0.71	6.34	0.96	18.84	0.89

Appendix 33: Hemoglobin polymorph percentage content of rainbow trout acclimated at 5 °C, Hypoxia, 8L:16D

Fish	C8	C7	C6	C5	C4	C3	C2	C1	A1	A2	A3	A4
85	1.35	8.55	8.07	10.72	26.86	14.33	1.68	0.36	5.30	0.84	20.0	0.84
86	1.07	6.92	9.07	9.23	25.07	15.84	0.77	0.46	6.92	1.53	16.15	0.61
87	1.08	6.62	9.39	8.91	26.86	15.66	0.96	1.32	10.72	1.20	16.98	0.48
88	0.57	7.50	7.84	11.02	25.91	13.29	0.56	1.13	3.97	1.02	20.45	0.68
89	0.61	7.65	8.47	10.20	30.20	14.28	0.51	1.12	6.02	0.61	21.63	0.20
90	0.60	5.06	10.0	11.80	29.27	11.68	0.24	1.44	12.04	0.36	17.71	0.24
91	0.88	4.42	9.73	7.25	26.54	16.99	0.88	0.88	13.45	1.32	17.70	0.88
92	1.20	8.31	9.63	10.84	26.50	14.82	1.44	1.20	4.82	1.20	18.79	1.20
93	1.22	7.89	7.66	11.11	25.55	13.44	1.0	1.0	5.77	1.33	22.22	1.77
94	0.89	8.20	7.05	7.69	31.15	14.36	0.38	1.28	5.0	1.02	22.18	0.38
95	0.69	8.04	7.81	10.92	26.43	14.02	0.46	1.15	5.86	0.92	22.75	0.80
96	1.04	7.08	8.54	11.98	26.14	12.70	1.35	1.14	7.08	1.56	22.91	0.52
$\bar{X}$	0.93	7.18	8.60	10.14	27.20	14.28	0.85	1.04	7.24	1.07	19.95	0.71
Sn-1	0.2680	1.2885	0.9502	1.5371	1.9231	1.4457	0.4546	0.3281	3.0855	0.3581	2.4212	0.4376
S^2	0.0718	1.6603	0.9028	2.3627	3.6985	2.0901	0.2066	0.1076	9.5205	0.1282	5.8625	0.1915
$\bar{Sx}$	0.0773	0.3719	0.2742	0.4437	0.5551	0.4173	0.1312	0.0947	0.8907	0.1034	0.6989	0.1263
U	1.10	8.0	9.20	11.11	28.42	15.20	1.14	1.24	9.20	1.30	21.49	0.99
L	0.76	6.36	8.0	9.16	25.98	13.36	0.56	0.83	5.28	0.84	18.41	0.43